SOCIAL, ECOLOGICAL, AND DEVELOPMENTAL INFLUENCES ON FRUIT AND INVERTEBRATE FORAGING STRATEGIES AND GUT MICROBIAL COMMUNITIES IN WHITE-FACED CAPUCHINS (*CEBUS CAPUCINUS*)

BY

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DISSERTATION

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ABSTRACT

Primates are challenged by spatiotemporal variation in resource availability, and a central question in biological anthropology is how primates compensate for seasonal variation in food resources by adjusting their foraging strategies. How primates respond to variation in invertebrate availability has rarely been the focus of studies of primate foraging ecology. This dissertation examines the role of insectivory in shaping foraging strategies, elucidates developmental differences in invertebrate foraging strategies, and investigates the role of the gut microbiome in mediating dietary changes in white-faced capuchins. White-faced capuchins (*Cebus capucinus*) are an instructive model for examining the influences of changes in both fruit and arthropod availability on foraging strategies, as they devote a mean of 44.4% of feeding and foraging time to fruit, 38.0% to invertebrates, and 1.2% to vertebrates.

A group of 20-22 white-faced capuchins was studied from January 2013 through January 2014 at La Suerte Biological Field Station in northeastern Costa Rica. Data was collected from individually recognizable adult and juvenile capuchins on diet (fruit, invertebrates, leaves, seeds, vertebrates, other), activity budget (feeding, foraging, traveling, resting, social, other), affiliative and agonistic interactions, nearest neighbor identity and distance, foraging subgroup size and spread, and geographic location at 2-minute intervals during 1-hour focal follows. Crown volume, diameter at breast height, number of food items in the crown, and average mass of five food items was collected for each tree in which the group fed for more than four minutes in order to assess patch productivity. Every two weeks, fruit resource availability was tracked using 25 100x4 meter phenology transects, and invertebrate resource availability was assessed using 10 composite insect traps and sweep net sites. Fecal samples were collected throughout the study

period (n=225). DNA was extracted from fecal samples, and the *COI* mtDNA and the v3-v5 region of the 16S rRNA genes were amplified and sequenced to identify invertebrates consumed and the gut microbial community structure.

The second chapter uses social network analysis to quantify group-level responses of white-faced capuchins to changes in food availability. The results indicate that increases in fruit abundance and decreases in patch density increase group cohesion (network density = 0.48±0.01 during periods of high abundance and patch density, network density = 0.40±0.07 during periods of low abundance and patch density), indicating that individuals may be decreasing group cohesion as fruit resources become less available in order to avoid feeding competition. Additionally, the abundance and distribution of invertebrate resources does not have a consistent effect on group cohesion, and the results suggest that capuchins do not see invertebrates as a uniform resource. In the third chapter of my dissertation, innovative molecular methods are used to identify the taxa of invertebrates present in the diet of white-faced capuchins and more closely investigates how animal prey foraging strategies are influenced by invertebrate availability, and the role of ontogeny on the development of foraging skills in capuchins. This chapter compares frequency with which DNA sequences assigned to specific Orders and Families of invertebrates are found in adult and juvenile feces, showing that juvenile capuchins are eating embedded, concealed, and highly mobile invertebrates, such as Gryllidae and Cercopidae, less often when compared with adults. Additionally, the results indicate that white-faced capuchins are consuming a greater diversity of arthropod prey than other New World monkey species, with the exception of squirrel monkeys, with 29 Orders, 90 Families, and 287 genera of invertebrates identified in their diet. Finally, chapter four examines how changes in fruit and invertebrate foraging behavior and dietary choice influence gut microbial community structure and function

in white-faced capuchins. This chapter shows that the relative abundance of several microbial genera have significant relationships with the minutes per hour spent feeding and foraging on several fruit species and invertebrate Families. In addition, these same fruit and invertebrate taxa have significant relationships with the relative abundance of predicted microbial metabolic functional pathways in the gut.

This dissertation presents a multi-level approach to studying white-faced capuchin foraging ecology, and the findings underscore the importance of looking beyond food abundance and distribution as the primary factors driving nonhuman primate foraging strategies. The results suggest that models of primate foraging strategies should include not only ecological and social information, but also individual-level factors such as physiology, personality, genetic traits, and commensal microbial relationships. The integrative multifaceted approach to primate foraging ecology in this dissertation provides a framework with which to begin to truly understand the complexity and plasticity of primate foraging strategies.

This dissertation is dedicated to my grandfather, I. Floyd Mallott, who knew I wanted to be an anthropologist before I did.

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CHAPTER 1

GENERAL INTRODUCTION

One of the central questions in biological anthropology is how primates balance the costs and benefits of group living, including intra- and inter-group feeding competition, predation avoidance, and access to reproductive partners (Wrangham, 1980; Van Schaik and Van Hooff, 1983; Terborgh and Janson, 1986; Sterck et al., 1997; Chapman and Chapman, 2000). The socioecological model posits that primate social behavior and patterns of spatial association are driven primarily by ecological variables and avoidance of feeding competition (Wrangham, 1980; Terborgh and Janson, 1986; Sterck et al., 1997; Snaith and Chapman, 2007). Previous studies of foraging strategies in primates have investigated the challenges faced by fruit- and leaf-eating primates in consuming resources that vary spatiotemporally in their availability, productivity, and nutritional content, indicating that primate group cohesion decreases when resources are available in fewer, smaller, and/or more scattered patches (Chapman and Chapman, 2000; Lynch Alfaro, 2007; Asensio et al., 2008, 2009; Aureli et al., 2008; Henzi et al., 2009; Sugiura et al., 2011; Schreier and Swedell, 2012). However, few studies have examined the role of insectivory in shaping primate foraging strategies, the influence of development on the costs and benefits of different prey foraging strategies, or how an individual's genetic makeup, levels of specific hormones, and commensal microbial relationships may contribute to differences in foraging strategies (Dawson, 1976; Peres, 1992; Gursky, 2000; Johnson and Bock, 2004; Agostini and Visalberghi, 2005; MacKinnon, 2006; Gunst et al., 2008, 2010; Haugaasen and Peres, 2009; Bogart and Pruetz, 2011; Melin et al., 2014; Mosdossy et al., 2015).

For many species of both larger- (humans, chimpanzees, gibbons, patas monkeys, macaques, mangabeys, bearded sakis, drills, guenons, woolly monkeys) and smaller-bodied (capuchins, tamarins, squirrel monkeys, titi monkeys) primates, animal prey, which is high in proteins and lipids, represents a major component of the diet (Bartlett, 2011; Bogart and Pruetz, 2011; Di Fiore et al., 2011; Jaffe and Isbel, 2011; Thierry, 2011; Swedell, 2011; Shaffer, 2013). Several authors have indicated that invertebrate resources are distributed differently in tropical forests when compared with fruit resources, and nonhuman primates may see arthropods as a more dispersed and, depending on the invertebrate taxa, less monopolizable resource (Robinson, 1986; Brien and Kinnaird, 1997; Isbell et al., 2013; Melin et al., 2014; Webster et al., 2014). Therefore, group cohesion is expected to decrease when primates are foraging on invertebrate resources when compared with fruit resources. However, other authors have suggested that cooperative foraging strategies may confer benefits when foraging for arthropod resources, in which case we would expect primate groups to be more cohesive during invertebrate foraging bouts than during fruit foraging bouts (Peres, 1992, 1993; Panger et al., 2002; Nadjafzadeh and Heymann, 2008; Haugaasen and Peres, 2009). Using cooperative foraging strategies, where individuals share information about food resources, are tolerant of co-feeders, and can flexibly use different foraging strategies, the rate at which individuals enter an occupied patch is expected to increase as resource availability and patch productivity increase (Vickery et al., 1991; Giraldeau and Caraco, 2000; Di Bitetti and Janson, 2001; Bicca-Marques and Garber, 2005). Cooperative foraging may increase patch detection rates and foraging success, even under conditions of reduced food availability if capture success increases as the number of co-foragers increases (Giraldeau and Caraco, 2000). However, as the number of co-feeders approaches the maximum number of individuals a patch can accommodate, additional joiners are expected to

encounter an overflocking cost, as they may join a resource after it has already been fully or nearly depleted (Vickery et al., 1991). Increasing our understanding of which conditions of invertebrate availability promote increased group cohesion may give us important insight into cooperative foraging behaviors in primates.

An understanding of prey foraging strategies in primates is essential, as the exploitation of high protein and fat foods such as insects, lizards, and small mammals has been argued to allow for the maintenance of the large brain volume required to integrate complex ecological information (location and productivity of resources) with complex social information (identity and number of individuals utilizing a resource) in decision-making (Garber and Bicca-Marques, 2009; Sussman and Garber, 2011). Our understanding of temporal (seasonal) and spatial (microhabitat) variation in invertebrate availability on primate foraging ecology is not well understood (McGrew, 2001). For example, *Chiropotes sagulatus* have been reported to devote between 1% and 36% of feeding and foraging time to invertebrates in any given month, and *Pan troglodytes* termites account for from 0% to 60% of total feeding and foraging time and rates of termite fishing are not correlated with with periods of low fruit availability (Bogart and Pruetz, 2011; Shaffer, 2013), but the factors influencing these fluctuations in invertebrate prey foraging are unknown. In tarsiers (*Tarsius spectrum*), Gursky (2000) found that more time was spent foraging for invertebrates during the dry season when invertebrate abundance was lower. In contrast, in galagos (*Galago senegalensis* and *G. crassicaudatus*), Harcourt (1986) found decreased or no change in the time spent foraging for invertebrates in the dry season. In squirrel monkeys, Stone (2007) found that increased time was spent feeding and foraging for invertebrates in the dry season, despite finding no seasonal variation in insect biomass. However, none of these studies directly measure the amount of prey items being consumed, limiting their

ability to ascertain the influence of variation in prey availability on prey foraging strategies. These studies suggest that invertebrate diversity and the availability of specific arthropod taxa may be more important to primate responses to seasonal changes in arthropod availability than overall invertebrate abundance.

There is evidence of seasonal variability in the Orders of arthropods consumed by prosimians, monkeys, and apes (Dawson, 1976; Harcourt, 1986; Isbell, 1998; Gursky, 2000; Stone, 2007a; Bogart and Pruetz, 2011; Deluycker, 2012; Mosdossy et al., 2015); however, only Gursky (2000) and Mosdossy *et al.* (2015) directly measured variation in the availability of specific Orders. By trapping invertebrates monthly using malaise and pitfall traps, she reported that the increased abundance of Orthopterans, Lepidopterans, and Hemipterans resulted in tarsiers foraging their efforts on these more abundant taxa (Gursky, 2000). However, she also found that Hymenopteran, Coleopteran, Isopteran and Arachnid consumption decreased in the wet season when these taxa were more available (Gursky, 2000). These data suggest that overall prey abundance, which may serve to increase foraging success, was important in tarsier food choice; however, increases in the availability of specific taxa of invertebrates also influences tarsier foraging strategies. Similarly, in a study of white-faced capuchins (*Cebus capucinus*) Mosdossy (2015) found significant positive correlations between caterpillar abundance and the rate of caterpillar consumption, and between non-caterpillar invertebrate abundance and the rate of consumption of non-caterpillar invertebrates. However, neither of these studies addressed availability or consumption of invertebrate taxa at lower taxonomic levels (i.e., Family or Genus), and Gursky (2000) measured the amount of time spent foraging for Orders of invertebrates, not the rate of consumption of each taxa. Integrating direct measures of variation in arthropod abundance such as the abundance and availability of specific Orders and Families of

invertebrates with behavioral observations of foraging success and social foraging strategies, combined with high-throughput sequencing to determine the invertebrates present in the diet of individual male, female, and juvenile capuchins, will allow us to further examine these questions regarding capuchin prey foraging behavior.

In addition to the influence of arthropod availability, developmental differences may play a large role in shaping nonhuman primate invertebrate foraging strategies. Studies have shown that in several nonhuman primate taxa juveniles are less efficient foragers, have difference food choices, or exhibit a smaller range of foraging techniques when compared with adults (Harrison, 1983; Watts, 1989; Agetsuma, 2001; Corp and Byrne, 2002; Hanya, 2003; Ganas and Robbins, 2004; Johnson and Bock, 2004). In green monkeys (*Cercopithecus sabaeus*), adult males spent a greater percent of their feeding time on flowers than juveniles (adult males – 6%, juvenile females and males –2%), adult females spend a lower percentage of the feeding time on fruit and invertebrates than juveniles (fruit: adult females – 25%, juvenile females – 30%, juvenile males – 31%; invertebrates: adult females – 4%, juvenile females – 8%, juvenile males – 7%) (Harrison, 1983). In this study, the author argued that differences in diet were likely due to differences in nutritional requirements in addition to priority of access to highly preferred foods such as flowers (Harrison, 1983). However, this study does not address whether or not these differences in time spent feeding were related to foraging efficiency, feeding rates, or differences in foraging skills. In two studies of Japanese macaques (*Macaca fuscata*), when compared with adult individuals, juvenile individuals had a lower feeding rate when foraging for fibrous foods (juveniles – 0.029-0.27 units/second; adults – 0.041-0.26 units/second), and focus their feeding efforts more on invertebrates (juveniles – 0-25% of feeding time in a given month vs. adults – 0-15%) and less on mature leaves (juveniles – 0-19% of feeding time in a given month vs. adults – 0-75%)

(Agetsuma, 2001; Hanya, 2003). Similarly, in hamadryas baboons (*Papio ursinus hamadryas*), Johnson and Bock (2004) found that juveniles focus their foraging efforts on foods for which their feeding efficiency is similar to that of adults.

In other nonhuman primate taxa, there is mixed evidence (Fragaszy and Boinski, 1995; Mackinnon, 1995; Agostini and Visalberghi, 2005; Stone, 2006, 2007b; Gunst et al., 2008, 2010; Bezanson, 2009) or little to no evidence (Post et al., 1980; Yamagiwa et al., 1991; Peláez et al., 2000; Prates and Bicca-Marques, 2008; Schmitt, 2010) for age-based differences in invertebrate foraging strategies. For example, in squirrel monkeys (*Saimiri sciureus*), there is no significant difference between juveniles and adults in the capture rate of invertebrates of any size (Stone, 2006, 2007b). The ability to process fruits with tough husks appears to be strength dependent, as small juveniles do not eat unopened fruits but there is no difference in handling time between larger juveniles and adults (Stone, 2006). However, juveniles forage for invertebrates in dead leaves more frequently and in palm foliage, indicating a difference in substrate preference for arthropod foraging (Stone, 2006). In spider monkeys (*Ateles belzebuth*) and woolly spider monkeys (*Lagothrix poeppigii*), Schmitt (2010) found few age-related differences in foraging behaviors. Though older juveniles in spider monkeys spent less time foraging than adults; however, there was no consistent developmental pattern in foraging budget differences between age-classes, nor were there significant differences between age-classes for fruit feeding rates (adults – 0.11±0.0078 fruits/sample vs. juveniles – 0.13±0.025 fruits/sample) (Schmitt, 2010). Similarly, in woolly spider monkeys, there were no age-related differences in the time spent feeding or foraging, foraging budgets, invertebrate capture rate, or fruit feeding rate (Schmitt, 2010). However, none of these three species relies heavily on extractive foraging techniques when foraging for invertebrates or forages extensively on concealed invertebrates.

Arthropods are frequently considered an easily acquired and readily available source of proteins and lipids for primates, and individuals with higher energetic requirements, such as juveniles and pregnant and lactating females, often rely more heavily on invertebrates in some species (Janson and Boinski, 1992; McGrew, 2001; McCabe and Fedigan, 2007). Embedded invertebrates, however, may not be readily available to all juvenile individuals, as juvenile primates not only must develop the necessary strength and motor skills to extract and process arthropods, but also must acquire the knowledge required to search for and detect concealed prey (Ross and Jones, 1999; Johnson and Bock, 2004; Gunst et al., 2010). Often, juvenile primates are acquiring animal prey items at the same rate as adults or appear to be using the same foraging strategies, but may be focusing their efforts on different substrates or surface invertebrates. However, studies of primate insectivory often lump all invertebrates into the same dietary category, despite the diversity of microhabitats, substrates, and life stages of invertebrates eaten by nonhuman primates. Without specific data on the taxa of prey being consumed, it is difficult to conclude whether or not juvenile individuals have a comparable diet to adults.

Studies of insectivory in nonhuman primates, however, have been limited by an inability to accurately identify the taxa of prey being consumed by individuals through direct observation. Generally, prey acquisition is rapid and in many studies researchers report that in 25-80% of captures the species of prey is scored as unknown (Terborgh, 1983; Peres, 1993; Nekaris & Rasmussen, 2003; Nafjafzahed & Heymann, 2008). DNA barcoding has been used successfully to assess the diet of wild primates (Bradley et al., 2007; Hofreiter et al., 2010; Pickett et al., 2012; Hamad et al., 2014; Mallott et al., 2015), as well as being used to identify invertebrate and vertebrate mitochondrial DNA found in the feces of bats, seals, and large cats (Deagle et al., 2005; Deagle and Tollit, 2006; Pons, 2006; Clare et al., 2009, 2011, 2014; Bohmann et al., 2011;

Zeale et al., 2011; Shehzad et al., 2012; Sedlock et al., 2014). DNA barcoding uses short segments (100-300bp) of highly variable mitochondrial DNA to identify species present in environmental samples. In nonhuman primates, this technique can be combined with behavioral observation to examine in more detail the specific taxa of invertebrates or vertebrates present in the diet. For example, DNA barcoding identified 11 Orders, 15 Families, and 12 genera of arthropods in the diet of saddleback tamarins (*Saguinus weddelli*) (Mallott et al., 2015), and 3-15 arthropod orders in the diets of squirrel monkeys (*Saimiri sciureus)*, white-fronted capuchins (*Cebus albifrons*), red woolly monkeys (*Lagothrix poeppigii*), equatorial sakis (*Pithecia aequatorialis*), red titis (*Callicebus discolor*), and spider monkeys (*Ateles belzebuth*) (Pickett et al., 2012). However, DNA barcoding does have limitations due to primer biases, amplification stocasticity, and lack of knowledge of gut passage rates. This technique does not allow us to assess the relative contribution of prey to an individual's diet, when a given taxa was ingested, or at what developmental stage (larval, adult) a given invertebrate was eaten (Bradley et al., 2007; King et al., 2008; Pompanon et al., 2012; Mallott et al., 2015). Additionally, DNA barcoding of invertebrate mtDNA in the feces of primates does have the potential to detect secondary predation (invertebrates present in the gut of the consumed invertebrate), unintentionally consumed invertebrates (invertebrates in fruit or on leaves consumed by primates), and environmental contamination (from soil, leaf litter, or eggs laid on the surface of fecal samples) (King et al., 2008; Hofreiter et al., 2010; Pompanon et al., 2012; Mallott et al., 2015). Thus, it can only be used to address general questions concerning which taxa may be part of primate diets and how frequently DNA from those taxa appear in an individual's feces.

An additional way in which nonhuman primates may respond to the challenges of fluctuating fruit and invertebrate resources and the resulting changes in nutrient consumption is

through commensal relationships with their gut microbiomes (Tremaroli and Bäckhed, 2012; Amato, 2013a; Amato et al., 2014a; Schnorr et al., 2014; Gomez et al., 2015). The gut microbiome plays an integral role in animal nutrition, digesting otherwise unavailable resources, providing substrates for nutrient metabolism in the gut, and increasing nutrient uptake and utilization in the gut (Cummings and Macfarlane, 1997; Hooper et al., 2002). In mammals, these nutrient degradation functions include releasing sugars from complex plant polysaccharides, such as xylan, cellulose, and pectin, and metabolizing amino acids (Hooper et al., 2002; Gill et al., 2006; Ley et al., 2008b). Mammals who have not evolved specialized physiological and morphological adaptations to process complex polysaccharides (e.g sacculated stomachs, enlarged hindguts, production of cellulases or chitinases, specialized dentition or masticatory apparatuses) may rely on gut bacteria to fulfill these roles (Hooper et al., 2002).

Several studies have shown that gut microbial community composition is correlated with dietary differences and the caloric value of the diet, defined as the amount of energy absorbed, is influenced by the gut microbiome (Ley et al., 2005, 2006a, 2008b; Turnbaugh et al., 2006; Hildebrandt et al., 2009; De Filippo et al., 2010; Wu et al., 2011; Claesson et al., 2012; Amato et al., 2013; Nelson et al., 2013). In humans, a study of the gut microbiomes of children in both Europe and Burkina Faso found the opposite relationship (De Filippo et al., 2010). Children in Burkina Faso had a diet that was higher in fiber content and had higher relative abundances of Bacteroidetes and Acinetobacter (57.7% vs. 22.4% and 10.1% vs. 6.7%) and lower relative abundances of Firmicutes and Proteobacteria (27.3% vs. 63.7% and 0.8% vs. 6.7%) compared with European children (De Filippo et al., 2010). These studies, along with other studies in both humans and other species, indicate that energy rich diets higher in animal protein and lipids are associated with gut microbial communities with relatively higher abundances of Firmicutes and

Proteobacteria, and that diets higher in complex carbohydrates and plant foods are associated with higher abundances of Bacteroidetes and Acinetobacter. As both fruits and insects vary greatly in their nutritional content both between and within taxa, nutrient availability and consumption may vary with spatiotemporal variation in food availability (Bell, 1990; Van Schaik et al., 1993; Chivers, 1998; Chapman et al., 2003; O'Driscoll Worman and Chapman, 2005; Rothman et al., 2008; Raubenheimer and Rothman, 2013). The gut microbiome may be an additional way in which primates buffer these dietary changes in response to changing food and nutrient availability (Tremaroli and Bäckhed, 2012; Amato, 2013a; Amato et al., 2014a; Schnorr et al., 2014; Gomez et al., 2015). However, few studies have investigated the interaction of variation in natural environments (including variation in diet) and gut microbiome variation in wild populations of primates (Amato et al., 2013, 2014a; b; Moeller et al., 2013a; Gomez, 2014; Gomez et al., 2015).

This dissertation integrates data from a 12-month observation study of white-faced capuchin social and foraging behavior and diet with ecological data on the availability of both fruit and invertebrate resources and molecular data on invertebrate prey and microbial taxa found in fecal samples. It examines how nonhuman primates respond to spatiotemporal variation in resource availability at three levels – how variation in social spacing patterns at the group-level allows individuals to decrease the effects of feeding competition or increase the benefits of cooperative foraging, the influence of development on acquisition of prey foraging strategies and individual variation in dietary choice, and the response of commensal gut microbial community structure and function to changes in diet.

White-faced capuchins are an instructive model for examining the social and ecological influences of both fruit prey availability primate foraging strategies. White-faced capuchins are

distributed from Honduras to Ecuador and have group sizes ranging from 5-30 individuals. *C. capucinus* devote 44.4% of feeding and foraging time to fruit, 38.0% to invertebrates, and 1.2% to vertebrates, and *C. capucinus* have been reported to spend up to 68.4% of feeding and foraging time exploiting animal prey (Buckley, 1983; Rose, 1994; Baker, 1998; Urbani, 2009; McKinney, 2010). Capuchins are unusual among non-ape primates in that they are characterized by a large brain volume to body size ratio (66.5cc/3.2kg); exploit embedded or concealed prey; consume colonial (ant larvae, termites, wasp larvae) and solitary arthropods (cicadas, caterpillars, katydids); actively hunt vertebrate prey (coati pups, squirrels, frogs, lizards, bird eggs); maintain group-based social traditions; fur rub with medicinal plants; and hunt and forage collectively; (Janson and Boinski, 1992; Rose, 1997; Baker, 1998; Panger et al., 2002; Perry et al., 2003; Hartwig et al., 2011). Given the increased metabolic costs of growing and maintaining neural tissue, highly-encephalized primates like capuchins, great apes, and hominins require a diet high in proteins and lipids, and, as arthropod and vertebrate taxa vary in their nutrient content, prey selection may play a critical role in their nutritional strategies (Bell, 1990; Janson and Boinski, 1992; Aiello and Wheeler, 1995; Gunst et al., 2010; Raubenheimer and Rothman, 2013). Capuchins have developed behavioral adaptions which may increase capture success when foraging for social invertebrates, including tapping on the surface of dead branches to locate ant colonies (Janson and Boinski, 1992). Collective hunting (>1 individual hunting the same prey item), collective invertebrate foraging, and food sharing have been documented in *C. capucinus*, and there is some evidence that several individuals acting collectively during foraging and hunting results in a greater payoff for all participants than solitary foraging or hunting (Rose, 1997, 2001; Panger et al., 2002; Rose et al., 2003). In white-faced capuchins, Rose (1997) reports that 81% of squirrel chases involve ≥2 individuals, and chases involving 3 adult males

had a higher capture success rate than chases involving 1 or 2 males (33% vs. 25%). However, the increases in the total number of participants did not increase capture success in this study, and there was very little evidence that participation in a chase increased access to the carcass, though 34% of chases may have involved some coordination including running on parallel or intersecting trajectories and surrounding squirrels, and this potentially coordinated behavior may have resulted in increased capture success (Rose, 1997; Rose et al., 2003). Collective foraging may result in shorter food search times, information sharing, and the ability to capture larger or multiple prey (Rose, 1997, 2001; Giraldeau and Caraco, 2000). Capuchins are reported to forage for invertebrates in foraging parties that move together through the understory, increasing foliage disturbance possibly resulting in prey flushing, which may increase hunting success, as has been reported for other species of primates including tamarins and Goeldi's monkeys (Peres, 1992; Porter and Garber, 2007; Nadjafzadeh and Heymann, 2008; Haugaasen and Peres, 2009; Isbell et al., 2013). In other instances, capuchin subgroups follow army ant columns to access prey flushed out by the insects (Panger et al., 2002). Although researchers report that Orthopterans, Hemipterans, Hymenopterans, Isopterans, and Lepidopterans are common prey items , an accurate assessment of the full complement of arthropod taxa consumed remains unclear because much of capuchin foraging occurs in microhabitats such as clusters of dead leaves, tree cavities, and other embedded substrates in which the ability of researchers to identify the prey consumed is extremely limited.

Several studies have investigated the influence of fruit availability and productivity on patterns of group cohesion and spatial association in capuchins (Phillips, 1995; Vogel and Janson, 2007; Vogel et al., 2007). Phillips (1995) reported a strong positive logarithmic relationship between foraging party size (# individuals feeding in the same tree) and the DBH of

fruiting trees in *C. capucinus*. Vogel and Janson (2007) examined the relationship between feeding competition, patch productivity and patch size in white-faced capuchins. These authors found that rates of aggression were higher in less productive patches and in patches with more co-feeders (Vogel and Janson, 2007). Thus, these studies are consistent in indicating that in the case of fruit feeding, a decrease in patch size results in a decrease in subgroup size and an increase in aggression in less productive food patches. However, the influence of seasonal changes in prey availability, type, or foraging strategies on white-faced capuchin social spacing, partner preferences, and the benefits of collective action, remains unknown.

I address the relationship between resource availability and behavioral, developmental, and physiological responses in three successive chapters. Chapter two of my dissertation examines the influence of availability of food resources, both invertebrate and fruit resources, on social spacing and group cohesion during foraging bouts. I integrate behavioral and ecological data, examining how proximity relationships at both the dyadic level and the whole group level change in response to the abundance and distribution of both fruit and invertebrate resources. Additionally, I investigate the similarities and differences in capuchin fruit and invertebrate foraging strategies. In the third chapter of my dissertation, I more closely investigate how animal prey foraging strategies are influenced by invertebrate availability, and development of foraging skills capuchins. I use both observational and DNA metabarcoding approaches to determine what taxa of arthropod are in the white-faced capuchin diet, and address how differences between adult and juvenile foraging choices relate to differences in prey detection and extractive foraging abilities. Finally, chapter four examines how changes in fruit and invertebrate foraging behavior and dietary choice influence gut microbial community structure and function in white-faced capuchins. In particular, I look at how time spent feeding and

foraging on specific taxa of arthropods and fruit are correlated with the relative abundance of microbial taxa and predicted metabolic functions.

Examining the effects of group cohesion, individual differences in dietary choice, and how commensal relationships with the gut microbiota impact host nutrition during fruit and prey foraging provide insights into the costs and benefits of cooperative and competitive foraging strategies in primates (Winterhalder, 1996; Hill, 2002; Bowles, 2006; Fehl et al., 2011; Apicella et al., 2012). In the case of early humans, it has been argued that cooperative behavior, including cooperative hunting and food sharing, played a vital role the shift to habitual meat and marrow consumption (Winterhalder, 1996; Hill, 2002; Layton et al., 2012). Though my dissertation addresses this question in one group of one species of primate, I aim to provide a multi-faceted framework for addressing the major question of how food resource availability impacted the evolution of primate foraging strategies more generally.

CHAPTER 2

HOW DO VARIATIONS IN FRUIT AND INVERTEBRATE AVAILABILITY INFLUENCE GROUP-BASED FORAGING STRATEGIES OF WHITE-FACED CAPUCHINS (*CEBUS CAPUCINUS*)

Abstract

Studies of the influence of fruit availability on social spacing in primates have indicated that increases in fruit abundance increase group cohesion, while increases in fruit patch density decrease group cohesion. Whether or not increasing group cohesion during periods of lower or more dispersed invertebrate abundance increases foraging success due to the benefits of cooperative foraging is less clear. To better understand how variation in both fruit and arthropod abundance and patch density influences patterns of spatial association in primates, a group of *C. capucinus* was followed for a 12-month period (841 hours of observation) at La Suerte Biological Field Station. Using instantaneous focal sampling, information on activity budget, diet, nearest neighbor distance, and feeding subgroup size was collected for adult female (n=5), adult male (n=4), juvenile (n=11-12), and infant (n=0-2) capuchins. Fruit availability was assessed at 2-week intervals using 25 100x4 meter transects. Arthropod availability was measured using canopy insect traps (n=10) and sweep nets (n=10) every 2-weeks. Social network density, average path length, and average weighted clustering coefficient were calculated to assess network cohesion during feeding and foraging bouts for each two-week period of ecological sampling. Network density, a measure of number of dyadic associations in the group, was significantly higher during periods of high fruit abundance (fruit availability index >10) and high patch density (I_δ<5, Morisita's index of dispersion) than during periods of

low fruit abundance and both high and low patch density (0.48±0.01 vs. 0.40±0.07 and 0.38±0.09). Additionally, network density was significantly higher during periods of high arthropod abundance (>175 invertebrates) and low patch density (I_δ>1.15, Morisita's index of dispersion) when compared with periods of low arthropod abundance and high patch density (0.50±0.07 vs. 0.43±0.09). Average path length (a measure of the degrees of separation between all possible dyads in the group) and average weighted clustering coefficient (a measure of how frequently an individual's frequent social partners are associated with each other) were not significantly influenced by fruit or arthropod availability. Additionally, capuchin foraging efficiency was neither significantly influenced by measures of patch productivity nor strongly influenced by variation in food availability, but feeding rates were significantly lower when fruit patch density was lower (22.0±22.9 vs. 15.5±14.4). These results indicate that *C. capucinus* are not modifying their patterns of social spacing in response to variation in fruit or invertebrate availability, and that, despite changes in group cohesion, capuchin foraging success did decrease with decreases in fruit patch density.

Introduction

Differences in group cohesion and patterns of social spacing in primates are likely the result of behavioral strategies to balance differences in the costs and benefits of group living (Wrangham, 1980; Van Schaik and Van Hooff, 1983; Terborgh and Janson, 1986; Sterck et al., 1997; Chapman and Chapman, 2000). In the context of feeding ecology, decreases in subgroup size and cohesion have been described as a strategy to more effectively exploit small and scattered food patches while maintaining a larger social unit necessary for effective predator, mate, or territory defense (Wrangham, 1980; Van Schaik, 1983; Van Schaik and Van Hooff,

1983; Clark and Mangel, 1986; Terborgh and Janson, 1986; Kinzey and Cunningham, 1994; Chapman and Chapman, 2000; Aureli et al., 2008). Previous studies have investigated the challenges faced by fruit and leaf-eating primates in consuming resources that vary spatiotemporally in their availability, productivity, and nutritional content, and suggest that increases in fruit abundance or productivity increase foraging subgroup size or spatial cohesion in species such as brown capuchins (*Sapajus apella nigritus*), spider monkeys (*Ateles geoffroyi*), chacma baboons (*Papio hamadryas*), and Japanese macaques (*Macaca fuscata*) (Chapman and Chapman, 2000; Lynch Alfaro, 2007; Asensio et al., 2008, 2009; Aureli et al., 2008; Henzi et al., 2009; Sugiura et al., 2011; Schreier and Swedell, 2012). However, for primates that spend a large percentage of their feeding and foraging time searching for animal prey, the specific set of conditions favoring an increase in spatial cohesion during prey foraging remains unclear (Janson, 1985a; Robinson, 1986; Peres, 1993; Nickle and Heymann, 1996). For example, in species such as moustached tamarins (*Saguinus mystax*) and wedge-capped capuchins (*Cebus olivaceus*), foraging in more cohesive groups results in increased capture success (Robinson, 1981; Peres, 1992). In contrast, in red-tailed monkeys (*Cercopithecus ascanius*), inter-individual distances increased when foraging on invertebrates compared with fruit, and the number of insect captures decreased when they were foraging with conspecifics (Bryer et al., 2013).

Social network analysis provides a strong analytical framework with which to evaluate the influence of food availability on spatial and social relationships at the level of the social group (Wey et al., 2008; Foster et al., 2012; Brent et al., 2013). Social network analysis can be used to integrate and compare data on social interactions, spatial proximity, foraging strategies, and feeding ecology of individuals across different temporal, behavioral or ecological contexts (Henzi et al., 2009; Jacobs and Petit, 2011; Sueur et al., 2011; Foster et al., 2012; Brent et al.,

2013). Its advantage over other measures of social affiliation include measuring social and proximity interactions not just at the dyadic level, but of all individuals in the group simultaneously. In the present study, we use three measures of social network structure – density (the number of actual associations between individuals in the group/number of possible associations between individuals in the group, where a higher density indicates that more individuals in the group are interacting with a greater number of individuals), average path length (the average number of edges between each pair of individuals in a network, where an edge is defined as an association between two individuals, and as the number of edges increases the degrees of separation between individuals increases), and weighted clustering coefficient (measure of how many of an individual's associates are associated with each other) – to assess changes in the cohesiveness of the network in response to temporal variation in fruit and invertebrate availability in a group of wild white-faced capuchins (*Cebus capucinus*).

White-faced capuchins are an instructive model for examining the influences of changes in both fruit and arthropod availability on group cohesion and social interactions. White-faced capuchins devote a mean of 44.4% of feeding and foraging time to fruit, 38.0% to invertebrates, and 1.2% to vertebrates, and have been reported to spend up to 68.4% of feeding and foraging time exploiting animal prey (Buckley, 1983; Rose, 1994; Baker, 1998; Urbani, 2009; McKinney, 2010). Nutritional values for invertebrate prey have been reported to be up to 22.9% crude protein and 61.5% lipids, making arthropods an important source of proteins and lipids (Bell, 1990; Janson and Boinski, 1992; Aiello and Wheeler, 1995; Gunst et al., 2010; Raubenheimer and Rothman, 2013). Capuchins are unusual among non-ape primates in that they are characterized by a large brain volume to body size ratio (66.5cc/3.2kg); exploit embedded or concealed prey; consume colonial (ant larvae, termites, wasp larvae) and solitary arthropods

(cicadas, caterpillars, katydids); and actively hunt vertebrate prey (coati pups, squirrels, frogs, lizards, bird eggs) (Janson and Boinski, 1992; Rose, 1997; Panger et al., 2002; Gunst et al., 2010; Hartwig et al., 2011).

Several studies of white-faced capuchins have investigated the influence of fruit availability and productivity on patterns of group cohesion and spatial association in capuchins (Phillips, 1995; Vogel and Janson, 2007; Vogel et al., 2007). The results indicate a decrease in patch size results in a decrease in subgroup size (from 6 individuals to 1 individual) and an increase in aggression in less productive food patches (Phillips, 1995; Vogel and Janson, 2007; Vogel et al., 2007; Crofoot, 2008). However, these studies have taken place in dry, seasonal forests in Guanacaste, Costa Rica (800-2600mm rainfall per year, mean=1473mm) (Fedigan and Jack, 2001) or in seasonal tropical rainforests on Barro Colorado Island, Panama (mean=2600mm rainfall per year) (Crofoot, 2008). At these sites, annual rainfall occurs primarily during a May-December wet season, and the abundance of fruit is highly correlated with rainfall. Additionally, at these sites, arthropod consumption increases during the dry season and one author argues that invertebrates that require extractive foraging techniques or processing prior to consumption are consumed by capuchins when other foods such as fruits or caterpillars are less available. (Mosdossy et al., 2015). In contrast, the northeastern region of Costa Rica is classified as an aseasonal tropical wet forest, with yearly rainfall averaging 3962mm (Sanford et al., 1994) and rainfall occurring throughout the year. As such, resource availability and use may not follow the patterns seen in more seasonal forests where white-faced capuchins have primarily been studied.

This study addresses five hypotheses about changes in white-faced capuchin social network cohesion and foraging strategies in response to changes in food availability. (**H1**)

During periods when food availability and patch density are high, nearest neighbor distances are expected to be smaller, foraging subgroup size expected to be larger, and network cohesion will be intermediate (measured as network density, average path length, and average weighted clustering coefficient). This is due to the fact under these conditions there are lower costs both to leaving a patch (the same or increased food intake and decreased time spent traveling and distance traveled) and to co-foraging (decreased aggression and the same or increased foraging efficiency). (**H2**) During periods when food availability is high and patch density low (increase in inter-patch distance), nearest neighbor distances are expected to be smaller, foraging subgroup size will be larger, and networks will be highly cohesive, as there are higher costs to leaving a patch and a low costs of co-foraging. (**H3**) During periods when patch density is high, but the amount of food per patch is limited to less than the amount required to satisfy all group members, nearest neighbor distances will be larger, subgroup size will be smaller, and networks will not be as cohesive, as there is a lower cost to leaving a patch and a higher cost to co-foraging. (**H4**) During periods when food is available in limited, dispersed patches (low food availability, low patch density), nearest neighbor distances will be larger, subgroup size will be smaller, and network cohesion will be intermediate, as there is a higher cost both to leaving a patch and to co-foraging. (**H5**) If white-faced capuchins are able to adjust their proximity networks during foraging to reflect changes in food availability and patch productivity, I expect that foraging efficiency (measured as a ratio of time spent feeding to time spent foraging in patches where the group is foraging for four or more minutes) and feeding rates (amount of grams ingested per unit time for fruit or number of invertebrates consumed per minute) will not differ across food availability conditions or be influenced by measures of patch productivity.

Materials and Methods

Study site and population description

Research was conducted from January 2013 through January 2014 at La Suerte Biological Field Station (LSBFS) in northeastern Costa Rica (10.445N, 83.784W). LSBFS contains approximately 300 ha of wet, lowland tropical forest and yearly rainfall during the study period at LSBFS was 3116 mm (rainfall data was collected daily with a rain gauge at the field site). Monthly rainfall ranged from 67 mm to 541 mm, with February, April and October having the lowest rainfall amounts, and March, July and November having the greatest monthly rainfall total (Table 2.1). There is an ongoing managed reforestation project at this preserve, and the forest includes 170 ha of advanced secondary forest and 130 ha of early secondary forest and regenerating pasture (Garber and Paciulli, 1997). LSBFS has a low density of predators, as apex predators are no longer present at the site, and capuchins at this site have low rates of intergroup encounters (0.0224 instances per hour, pers. obs.). Four groups of *C. capucinus* are present, one of which is habituated. *Ateles geoffroyi* and *Alouatta palliata* also are present at the site. The group of habituated, individually recognizable white-faced capuchins that were used for the study contained 21-22 individuals (4 adult males, 5 adult females, 11-12 juveniles, and 0-2 infants). All data collection methods were approved by the University of Illinois IACUC, La Suerte Biological Field Station, MINAET, and SINAC.

Behavioral data

With the help of two field assistants, I conducted simultaneous 1-hour instantaneous focal animal samples (2-min interval), using individually recognizable adult males, adult females, and juveniles as focal subjects, 5 days a week (20 days/month) for 12 months. In total, I collected

841 hours of quantitative behavioral data during 1341 hours of observation on 237 days. Behavioral data collection was biased towards the morning (66.4% of quantitative data was collected between 0530-1100). Information was collected on activity budget (feeding – activity consuming food items; foraging – manipulating or searching for food items, including localized movement in the crown of a potential feeding site; traveling – movement between or within the crowns of trees which was not for the immediate purpose of obtaining a food reward or engaging in a social activity; resting – period of inactivity; social – engaged in any affiliative or agonistic interaction with at least one other individuals; other), diet (ripe fruit, unripe fruit, invertebrates, vertebrates, flowers, leaves, other), feeding rate (number of whole food items consumed during the 2-min interval – if an entire food item was not consumed, it was scored as 0.25, 0.5, or 0.75 based on the proportion consumed), social interactions (including affiliative, aggressive, and submissive behaviors). Diet was calculated as the percent of total feeding and foraging records.

Data was collected on nearest neighbor identity and distance, as well as the number and identity of individuals feeding or foraging in the same patch (tree or adjacent trees with continuous canopy of the same species). Dependent infants (individuals <6 months still being carried by the mother) were never noted as the nearest neighbor of their mother. The probability of nearest neighbor dyads being of a particular age-sex combination did not deviate from the expected probability based on group composition (χ^2 test, all $p>0.05$, Table 2.2). Nearest neighbor distances were straight-line distances estimated to the nearest meter for distances under 15 m and to the nearest five meters for distances >16 m. Observer reliability for nearest neighbor distance estimation was tested periodically using a meter tape. The results indicated that all observers were accurate to within a half-meter. Subgroups of varying composition (including adults, subadults, and juveniles) were combined when subgroup size (the number of individuals

feeding or foraging in the same patch) was calculated. Dependent infants were excluded from subgroup analyses. An average of 1.3 hours of feeding and foraging data were collected per day, with 268.6 total hours of feeding and foraging recorded (40% of capuchin activity budget). The identity of both the focal and the nearest neighbor was known for 3374 of 8051 (41.9%) feeding and foraging records and 1898 of 2199 (86.3%) grooming records.

Fruit patch productivity

For all trees in which focal individuals fed or foraged in for at least four consecutive minutes (N=311), the tree was tagged with the date and ID#. During that same week, I or a field assistant recorded: crown diameter (measured at the widest diameter with a meter tape on the ground), estimated crown height, and crown shape (cone, cylinder, cube, sphere); a phenology score (percentage of the crown containing ripe fruit: 0-25% = 0.25, 25-50% = 0.5, 50-75% = 0.75, >75% = 1), and fruit score (number of ripe fruits, unripe fruits, and flowers in the crown: 0-10 = 0.01, >10-25 = 0.25, >25-50 = 0.5, >50-100 = 0.1, >100 = 1) (see Crofoot, 2008 for a similar method). Crown volume was calculated from crown size measurements using standard forestry formulas for each crown shape (Coder, 2000), scored as cone, cylinder, paraboloid, and spheroid. For each feeding patch (tree or adjacent trees with continuous canopy of the same species), fruit mass ($\sum(average\ weight\ of\ 5\ fruits/flowers \times fruit\ score)$) and a patch productivity index were calculated (PPI = $\sum(crown\ volume \times fruit\ mass \times phenology\ score)$).

Fruit abundance

Twenty-five random 100x4 meter line-transects (total area sampled = 0.01 km^2) were surveyed every two weeks to track detailed changes in fruit availability. The number of fruiting tree species that the capuchin group had been observed consuming at least once in the preceding two weeks was noted for each transect. For each fruiting and flowering tree, the same metrics used to assess patch productivity were recorded (see above). A fruit abundance index (FAI = $\frac{\sum(crown\ volume \times fruit\ mass \times phenology\ score)}{total\ crown\ volume\ sampled}$) was calculated for each transect every two weeks. On average, 16.2±5.2 (range 10-27) trees were sampled across all transects per two-week period in the same area, which comprised 0.5% of the group's total home range (2.07 km^2). All trees sampled on transects were recorded as part of the group's diet at least once in the preceding two weeks.

Invertebrate abundance

Arthropod availability was assessed by bi-monthly collections using 10 Composite Insect Traps (Russo et al., 2011) placed at randomly chosen geographic coordinates within a 20.2 ha area of the study group's home range, hoisted on a pulley system to 5-15 m above the ground. Canopy traps, measuring 0.5x1.5 m, were hung for 72 hours every two weeks. Sweep netting (height of 1.5 m with a 0.5 m diameter net) was conducted for 1 minute at trap locations between 0700 and 1300 every two weeks, coinciding with days when arthropods were collected from the canopy traps. On average, we swept 30 times per minute. We avoided sweep netting during heavy rain, but it was not always possible to avoid collection during light rain and rarely possible to avoid sweeping when vegetation was wet. Arthropods from each trap and sweep netting sample were counted and identified to Family using classification keys and reference books in the library at LSBFS.

Resource dispersion

Morisita's index of dispersion ($I_\delta = q\frac{\sum_{i=1}^{q} n_i(n_i-1)}{N(N-1)}$, where q=number of transects or trapping locations, n_i=number of trees in a transect or invertebrates in a trapping location, and N=total number of trees or invertebrates sampled in a two-week period) (Morisita, 1959) was used to measure the dispersion of fruit and invertebrate resources. For this index, I_δ=1 when resources are randomly distributed, I_δ<1 when resources are uniformly distributed, and I_δ>1 when resources have a clumped distribution, with the patch density being negatively correlated with I_δ. Morisita's index of dispersion is independent of quadrat size and sample size (Morisita, 1959).

Social network construction

Proximity association matrices were calculated in SOCPROG 2.5 (Whitehead, 2009) from nearest neighbor data using the half-weight index and were weighted by nearest neighbor distance. Proximity association matrices were calculated from all behavioral records, fruit feeding and foraging bouts, and invertebrate feeding and foraging bouts for each ecological sampling period. Network density, average path length (or average distance), and average weighted clustering coefficient were calculated in UCINET 6 (Borgatti et al., 2002) for all matrices.

Statistics

ANOVAs were used to assess the influence of periods of high and low fruit and invertebrate abundance and dispersion on nearest neighbor distances, the number of individuals

in a feeding patch, rates of aggression, and social network parameters, and significant differences between conditions were identified using a Tukey test. Mixed effects linear models were also used to assess the influence of DBH, crown volume, patch productivity, resource abundance, and resource dispersion on foraging efficiency and feeding rates. Individual was included in both ANOVAs and linear models as a random effect to control for pseudoreplication. T-tests were used to compare foraging efficiency and the number of individuals co-foraging in a patch between invertebrate and fruit feeding and foraging records. Paired t-tests were used to compare network measures between fruit and invertebrate feeding and foraging contexts. Pearson correlations were used to test the relationship between patch productivity and the number of co-foragers in a patch. All statistical analyses were carried out in R (R-project.org).

Results

Diet, Foraging behavior, food availability, and patch size

The activity budget of the study group was feeding=12.6%, foraging=26.3%, resting=17.8%, social=13.8%, and traveling=29.5%, and the diet, based on feeding and foraging time, was ripe fruit=47.2%, unripe fruit=0.7%, flowers=1.2%, leaves=0.5%, invertebrates=49.8%, and other=0.6% (n=18905 individual activity records, other includes vertebrates and seeds) (see Appendix A and B). On average, individuals spent 23.2 minutes per hour feeding and foraging (range=17.5-29.6). Foraging efficiency (minutes per hour feeding/minutes per hour foraging) was 0.4769 for all food types, but varied by food type. Foraging efficiency was significantly higher during fruit foraging bouts compared with invertebrate foraging bouts (1.21±1.25 vs. 0.26±0.31, $t_{296.89}$=11.24, $p<0.001$).

The fruit availability index averaged 10.63 (range=0.35-41.40) over the course of the study, and was highest from November through April and September (Figure 2.1). Invertebrate abundance averaged 187.4 (range=69-328) insects captured per trapping period, with peaks in April, June, August and November (Figure 2.1). These data indicate that the period from April through September containing the highest abundance of both invertebrates and ripe fruits. However, neither fruit abundance nor arthropod abundance were significantly correlated with rainfall during the two-week ecological sampling period (all $p>0.05$). Rates of aggression did not differ significantly between the four conditions of fruit and invertebrate availability (all $p>0.05$).

Nearest neighbor distance and number of co-foragers in a patch

Fruit availability had a significant effect on nearest neighbor distance ($F_{3,16301}=12.33$, $p<0.001$), with periods of lower fruit abundance (FAI<10) and higher patch density ($I_\delta<5$, Morisita's index of dispersion) having significantly smaller nearest neighbor distances (7.28±6.67 m vs. 8.08±7.39 m, 8.25±7.29 m, and 8.46±6.89 m) than all other fruit availability conditions (Table 2.3 and Figure 2.2). This is contrary to the predictions of all hypotheses that periods of lower abundance would have higher nearest neighbor distances. Arthropod availability also had a significant effect on nearest neighbor distance ($F_{3,16301}=7.18$, $p<0.001$). Periods of lower invertebrate abundance (<175 arthropods) and higher patch density ($I_\delta<1.15$, Morisita's index of dispersion) had smaller nearest neighbor distances than periods of higher invertebrate abundance (>175 arthropods) and lower patch density ($I_\delta>1.15$, Morisita's index of dispersion) (Table 2.3 and Figure 2.3). Periods of high arthropod abundance (>175 arthropods) and high patch density ($I_\delta<1.15$, Morisita's index of dispersion) had smaller nearest neighbor distances than periods of low arthropod abundance and low patch density (Table 2.3 and Figure 2.3).

Under conditions of low patch density, increasing arthorpod abundance increases nearest neighbor distances, while under conditions of high patch density, increasing arthropod abundance decreases nearest neighbor distances. These results do not support hypotheses two or three that periods of lower abundance and higher patch density would have high nearest neighbor distances, but do support hypotheses one and four that periods of higher abundance and higher patch density would have lower nearest neighbor distances. However, even though nearest neighbor distances varied significantly between periods of high and low arthropod and fruit availability, with periods with less dispersed resources having smaller nearest neighbor differences, these differences are likely not ecologically significant, as they are differences of less than a meter (Table 2.3).

The average number of individuals feeding and foraging simultaneously in a patch was 2.49±1.87, with fruit feeding and foraging bouts having significantly, but minimally, higher numbers of individuals than invertebrate feeding and foraging bouts (2.99±2.27 vs. 2.03±1.24, $t_{2128.1}$=-14.08, $p<0.001$). Subgroup size was significantly positively correlated with both crown volume and DBH, but not patch productivity, of feeding trees ($r=0.246$, $p<0.01$; $r=3.17$, $p<0.01$; $r=0.010$, $p=0.83$). There was no significant effect of fruit availability on the number of individuals in a patch (all $p>0.05$) (Table 2.3), indicating that, for fruit foraging, none of our hypotheses were supported. Invertebrate availability did have a significant effect on the number of co-foragers in a patch ($F_{3,1589}=3.02$, $p=0.03$), with periods of high arthropod abundance and high patch density having fewer co-foragers than periods of high arthropod abundance and low patch density (3.35 vs. 3.71) (Table 2.3 and Figure 2.4). These results support hypotheses two and four, but not one and three. It appears that variation in invertebrate patch dispersion, measured using Morisita's index of dispersion, has a stronger influence on subgroup size than

invertebrate availability, as periods with higher arthropod availability do not have larger subgroup sizes.

Proximity networks

Fruit abundance and fruit patch density significantly influenced network density, a measure of how many dyadic associations there are in a group ($F_{3,15}$=4.14, p=0.025). Periods of low fruit abundance and both high and low patch dispersion having a significantly lower network density than period of high fruit abundance and high patch density (0.40±0.07 and 0.38±0.09 vs. 0.48±0.10) (Table 2.3 and Figure 2.5), which is contrary to the predictions of all hypotheses. Fruit abundance and fruit patch density did not significantly influence average path length, a measure of how tightly all individuals are connected in the network, or average weighted clustering coefficient, a measure of the average gregariousness of individuals in the group (all p>0.05) (Table 2.3). Arthropod abundance and patch density did significantly influence network density ($F_{3,21}$=4.24, p=0.02), with periods of high abundance and more clumped arthropod patches having significantly higher network density than periods of low abundance and more evenly distributed arthropod patches (0.50±0.07 vs. 0.43±0.09) (Table 2.3 and Figure 2.6). This data suggests that decreasing invertebrate abundance decreases network density, consistent with the predictions of hypotheses two and three. However, hypothesis four predicted that periods with low invertebrate abundance and high invertebrate patch density would have intermediate network densities. Our data did not support this, as these periods have the lowest network densities. There were no significant differences in network measures between fruit and invertebrate feeding and foraging contexts (all p>0.05) (Table 2.4).

Foraging efficiency and feeding rates

Foraging efficiency during fruit feeding and foraging bouts increased as fruit resource patchiness increased (t_{242}=2.98, p=0.003) (Figure 2.7), but was not influenced by fruit abundance (p>0.05). Foraging efficiency during invertebrate feeding and foraging bouts did significantly increase as arthropod abundance increased (t_{282}=2.55, p=0.01), but was not influenced by invertebrate patch distribution (Figure 2.6). In clumped fruit and arthropod patches individuals spent an average of 0.25±0.29 minutes feeding per minute foraging, whereas in dispersed patches individuals spent an average of 0.27±0.33 minutes feeding per minute foraging. These results do not support hypothesis 5, which predicted that foraging efficiency would remain constant across food availability conditions when network cohesion was variable across conditions. During fruit feeding, there was no significant effect of the DBH, total crown volume, or patch productivity index on foraging efficiency when controlling for the identity of individual capuchins (all p>0.05). Overall, in our study group foraging efficiency was more influenced by fruit distribution and arthropod abundance, being positively correlated with both, than fruit abundance or arthropod patch density.

Fruit dispersion had a significant influence of fruit feeding rates (t_{57}=-2.01, p=0.049), with feeding rates decreasing as fruit patch density became lower. Fruit abundance, however, did not have a significant effect on fruit feeding rates (p>0.05). These results indicate that capuchins may be consuming the fruit available in a patch less rapidly when inter-patch distances and travel costs between patches decrease, contrary to the predictions of hypothesis five. Neither invertebrate abundance nor dispersion had a significant influence on invertebrate feeding rates (both p>0.05). These results, combined with the social network analysis, indicate that the fluctuations in group cohesion, specifically network density, as a response to changes in

invertebrate availability may allow capuchins to maintain similar feeding rates across different conditions of arthropod availability, as predicted by hypothesis five.

Discussion

In this study we addressed questions concerning the influence of fruit and invertebrate availability on white-faced capuchin foraging strategies. By examining how both resource abundance and distribution influenced foraging behaviors using social network analysis, we examined the individual the effects of insect and fruit patch productivity and patch distribution on white-faced capuchin foraging strategies. Previous studies of the relationship between food availability and primate foraging strategies have argued that as fruit abundance increases, group cohesion increases, and that as fruit patch density increases, group cohesion decreases (Chapman and Chapman, 2000; Lynch Alfaro, 2007; Asensio et al., 2008, 2009; Aureli et al., 2008; Henzi et al., 2009; Sugiura et al., 2011; Schreier and Swedell, 2012). However, previous research does not address the influence of the availability of invertebrate resources on social spacing, and few studies examine both food abundance and distribution of invertebrate resources. Given that white-faced capuchins exploit a diet principally composed of invertebrates and ripe fruits, we tested five hypotheses.

The first hypothesis, that nearest neighbor distances would be smaller, foraging subgroup size would be larger, and network cohesion would be intermediate when food was distributed in dispersed, productive patches, was not supported during fruit exploitation. Periods with high fruit abundance and patch density had intermediate nearest neighbor distances (8.25±7.29 m vs. 8.46±6.89 m, 8.08±7.39 m, and 7.28±6.67 m) and cohesive social networks (network density of 0.48±0.01 vs. 0.41±0.03, 0.40±0.07, and 0.38±0.09), but subgroup sizes were smaller not larger

(3.44±1.71 vs. 3.32±1.72, 3.59±1.96, and 3.4±2.05). Hypothesis one was also partially supported for periods with high invertebrate abundance and patch density, as nearest neighbor distances were lower (7.76±6.69 m vs. 8.41±7.63 m, 8.27±7.28 m, and 7.97±6.96 m), but subgroup sizes were actually smaller (3.35±1.78 vs. 3.71±1.94, 3.41±1.66, and 3.44±1.95) and network density was lower (0.39±0.07 vs. 0.50±0.07, 0.45±0.09, 0.43±0.09). These invertebrate results are in contrast to the findings of a study of hamadryas baboons, where group cohesion was higher when groups were foraging on abundant, low density resources (palm nuts) (Schreier and Swedell, 2012). However, this is similar to our results for capuchin social spacing during fruit exploitation (Schreier and Swedell, 2012).

The second hypothesis, that periods with productive, clumped patches will have smaller nearest neighbor distances, larger foraging subgroup sizes, and highly cohesive networks was not supported in fruit foraging contexts. During these ecological conditions, capuchins had the largest nearest neighbor distances (8.46±6.89 m) and smallest subgroup sizes (3.32±1.72). Additionally, when examining group cohesion using social network metrics, network cohesion was intermediate (network density = 0.41±0.03, average path length = 1.66±0.09, average weighted clustering coefficient = 0.17±0.06) during periods of high fruit abundance and low fruit patch density, contrary to predictions. This is in contrast to existing data for Japanese macaques that indicates that subgroup sizes were large and nearest neighbor distances were small when foraging on highly abundant, clumped fruit resources (Sugiura et al., 2011). In this strudy, hypothesis two was only partially supported when examining invertebrate foraging contexts. Subgroup sizes were higher (3.71±1.94) and network density was higher (0.50±0.07), but nearest neighbor distances were also higher (8.41±7.63 m).

The third hypothesis was partially supported with regard to both fruit and invertebrate exploitation. During periods when fruit was available in less productive but more dispersed patches, nearest neighbor distances were smaller (7.28±6.67 m vs. 8.46±6.89 m, 8.25±7.29 m, and 8.08±7.39 m), contrary to the predictions, but subgroups sizes were smaller (3.4±2.05 vs. 3.32±1.72, 3.44±1.71, and 3.59±1.96), consistent with predictions. However, social network connectivity was low (network density = 0.38±0.09 vs. 0.41±0.03, 0.48±0.01, and 0.40±0.07), suggesting that group-level cohesion was decreasing as fruit abundance decreased and density increased. During periods when invertebrates were available in less productive but more dispersed patches, nearest neighbor distances were smaller (7.97±6.69 m) and subgroup sizes were larger (3.44±1.95), but network density was intermediate (0.43±0.09). This partially supports the predictions of hypothesis three, and appears to reflect the fact that capuchins may not be influenced the same way by changes in invertebrate distribution and abundance as they are by changes in fruit availability. Similar results have been supported in other studies of white-faced capuchins and, as well as in moustached tamarins, where it was been suggested that foraging in a cohesive group during insectivory increases capture success (Peres, 1992; Rose, 1997; Panger et al., 2002), and it is likely that capuchins are benefited by cooperative foraging behavior regardless of invertebrate abundance when foraging for evenly distributed arthropod resources.

During periods when both resource abundance and density were low, hypothesis four predicted that nearest neighbor distances would be larger, subgroup sizes smaller, and social networks would not be cohesive. This hypothesis was not supported during fruit exploitation. During periods of low fruit abundance and patch density nearest neighbor distances were smaller (8.08±7.39 m) and foraging subgroup sizes was larger (3.59±1.96). In addition, network density,

average path length, and average weighted clustering coefficient all had intermediate values (0.40±0.07, 1.63±0.12, and 0.18±0.07). Hypothesis four was partially supported for invertebrate foraging contexts. During periods of low arthropod abundance and patch density, subgroup size was smaller (3.41±1.66), and nearest neighbor distance was larger (8.27±7.28 m). However, network density was intermediate (0.45±0.09). This consistent with a study of brown capuchins (*Sapajus apella*) and squirrel monkeys (*Saimiri ustus*), which suggested that the benefits of prey flushing are higher in periods of lower invertebrate abundance and density, leading to increased group cohesion and foraging group size (Haugaasen and Peres, 2009). However, there may be a level of arthropod availability below which cooperative foraging is no longer beneficial for capuchins.

Hypotheses 1-4 were not supported for frugivory. This is in contrast to the results of other capuchin studies (Phillips, 1995; Vogel, 2004; Lynch Alfaro, 2007), which found that foraging subgroup sizes and nearest neighbor distances decrease as food availability decreases. Phillips [1995] suggested that white-faced capuchins are changing their grouping patterns based on the dimensions of the food patch, not the productivity of food resources within a patch, in part to maintain consistent inter-individual distances. Phillips [1995] reported a strong positive logarithmic relationship between foraging party size (# individuals feeding in the same tree) and the DBH of fruiting trees in white-faced capuchins. However, this relationship does not take into account the number of fruit patches or their distribution in the environment, simply the size of individual patches, which is not a measure of how much food is in the patch. We also found strong positive correlations between crown volume and DBH and the number of co-foragers in a patch, but did not find that increases in fruit abundance or patch productivity significantly increased subgroup size, decreased nearest neighbor distances, or increased network cohesion.

Previous studies of changes in capuchin social spacing in response to changes in fruit availability either looked solely at measures of availability in individual patches, which may not reflect the overall availability of food in the environment (Phillips, 1995; Vogel, 2004), or did not measure food availability directly or consider patch density in addition to abundance (Lynch Alfaro, 2007). Additionally, the number of co-foragers in a patch does not necessarily take into account proximity of individuals, and, therefore, may strongly be tied to patch size, not productivity. By using data on both abundance and distribution of fruit resources with social network analysis, which takes into account the strength and frequency of all possible dyadic interactions in the group weighted by proximity, our results do not support previous findings or the predictions of the socioecological model in regards to how white-faced capuchin group cohesion was affected by changes in fruit availability.

When examining the response of patterns of social spacing to changes in invertebrate abundance and distribution, no hypotheses were fully supported. Several studies of invertebrate foraging in primates have assumed invertebrates are a homogenously distributed resource (Robinson, 1986) and, at least for some invertebrates, availability is fairly continuous over the year [Brien & Kinnaird, 1997; Isbell et al., 2013; Melin et al., 2014; Webster et al., 2014; but see Fragaszy & Boinski, 1995; Tennie et al., 2014]. However, data from a recent study of white-faced capuchins in Santa Rosa indicates consistent evidence of strong seasonal changes in arthropod availability, particularly of ants, weevils, cockroaches, and caterpillars (Mosdossy et al., 2015). Moreover, capuchins are targeting specific invertebrate taxa, such as ant and wasp larvae, termites and caterpillars, that are not distributed evenly through space and time (Mosdossy et al., 2015; Mallott et al., 2016). The challenges primates face in exploiting patchily distributed but highly productive invertebrate nests, finding and extracted concealed and

embedded prey, foraging for highly mobile prey, and opportunistic consumption of invertebrates (Janson and Boinski, 1992; Peres, 1992; Panger et al., 2002; Nekaris and Rasmussen, 2003; Haugaasen and Peres, 2009; McGrew, 2014; Tennie et al., 2014; Mosdossy et al., 2015). Patterns of social spacing that increase capture success during one type of prey foraging may decrease it during foraging for other arthropod taxa. In this regard, when foraging for Lepidoptera and other highly mobile prey, foraging in cohesive groups may increase capture success, and we would expect to see smaller nearest neighbor distances, larger foraging subgroup sizes, and high network cohesion (Peres, 1992). In contrast, when foraging for clumped resources such as wasp nests, it may be more beneficial to be foraging in smaller subgroups, increase nearest neighbor distances, and decrease network cohesion. The inconsistency of support for our predictions may be due to the fact that, during many points during the year, capuchins are exploiting multiple taxa of invertebrates that require vastly different foraging strategies. Thus, future studies of invertebrate prey foraging strategies and social spacing during prey foraging would benefit from the addition of information on the microhabitats and substrates being exploited, as well as the specific taxa of prey being targeted in a given period of time.

Hypothesis five predicted that foraging efficiency and feeding rates would not differ across different food availability conditions or be influenced by patch productivity. While foraging efficiency was not influenced by fruit patch abundance, it increased as fruit patch density decreased and invertebrate resource abundance increased. However, foraging efficiency may not be a robust predictor of foraging success. Feeding rates did not differ across conditions of invertebrate availability nor were they influenced by changes in fruit abundance, feeding rates were lower as fruit patch density decreased (high density: 22.0±22.9 food items/two minutes, low density: 15.5±14.4 food items/two minutes). Even though capuchins modified their social

spacing in response to changes in fruit availability, and, to a lesser extent, in response to changes in invertebrate availability, some indicators of foraging success were still variable. Other studies of the effects of proximity to other individuals on foraging success in capuchins have mixed conclusions (Robinson, 1981; Janson, 1985a). In wedge-capped capuchins, Robinson [1981], found that invertebrate capture success was lower when an individual's closest neighbor was less than 2m away, compared with a nearest neighbor 3-4 m away. In contrast, in black-horned capuchins, Janson [1985] found that a more central location within the group resulted in both in smaller nearest neighbor distances and increased foraging success for both fruit and invertebrates. Taken together, the results of our study are not consistent with theories that argue that primates respond to changes in food resources by changing patterns of social spacing in order to minimize the costs of decreased foraging success when fruit and invertebrate resources are less abundant.

The results of our study indicate that capuchins do not increase cohesion at the group-level in response to increases in fruit abundance or decreases in fruit patch density during periods of low fruit abundance. Additionally, the relationship between group cohesion and invertebrate availability remain unclear. While changing group cohesion is one way in which primates buffer changes in food availability, other factors, such as kin relationships, age- and sex-class, and/or dominance rank may also play a role in determining to what extent primate foraging success is lowered when food resources are less available. Studies of the ecological factors that influence social network structure, including both fruit and invertebrate availability, have the potential to increase our understanding of group-level processes and the evolution of social group structure in nonhuman primates.

Tables and Figures

	Rainfall (mm)
January	152
February	103
March	541
April	67
May	214
June	251
July	469
August	315
September	224
October	98
November	351
December	185
January	146

Table 2.1. Monthly total rainfall at La Suerte Biological Field Station from January 2013 through January 2014.

	Adult female		Adult male		Juvenile				
	Observed	Expected	Observed	Expected	Observed	Expected	n=	Chi-square	p
Adult female	20.4%	20%	26.4%	20%	53.2%	55%	7698	0.02	0.989
Adult male	33.2%	25%	19.0%	15%	47.8%	55%	4843	0.05	0.977
Juvenile	33.1%	25%	15.9%	20%	51.0%	55%	3143	0.04	0.981

Table 2.2. Observed versus expected proportion of nearest neighbor dyads of each age-sex class during the behavioral study.

	Fruit				Invertebrates			
	High abundance, Clumped patches	**High abundance, Dispersed patches**	**Low abundance, Clumped patches**	**Low abundance, Dispersed patches**	**High abundance, Clumped patches**	**High abundance, Dispersed patches**	**Low abundance, Clumped patches**	**Low abundance, Dispersed patches**
Nearest Neighbor Distance	8.46±6.89*	8.25±7.29*	8.08±7.39*	7.28±6.67*	8.41±7.63*	*7.76±6.96**	*8.27±7.28**	7.97±6.96*
Subgroup size	3.32±1.72	*3.44±1.71*	3.59±1.96	*3.4±2.05*	*3.71±1.94**	3.35±1.78*	*3.41±1.66*	3.44±1.95
Network Density	0.41±0.03	0.48±0.01*	0.40±0.07*	0.38±0.09*	*0.50±0.07**	0.39±0.07	0.45±0.09	*0.43±0.09**
Average Path length	1.66±-0.09	1.56±0.15	1.63±0.12	1.70±0.15	*1.54±0.11*	1.69±0.13	*1.59±0.11*	*1.60±0.16*
Weighted clustering coefficient	0.17±0.06	0.15±0.06	0.18±0.07	0.19±0.07	*0.17±0.07*	0.17±0.05	*0.15±0.03*	*0.16±0.08*

Table 2.3. Average nearest neighbor distances, number of co-foragers in a patch, network density, network path length, and weighted clustering coefficient under all food resource availability conditions. Italicized numbers support the predictions. Starred numbers are significantly different from at least one other condition.

	Fruit	Invertebrate
Network density	0.245 (±0.078)	0.248 (±0.053)
Average path length	2.173 (±0.320)	2.137 (±0.243)
Average clustering coefficient	0.269 (±0.153)	0.312 (±0.100)

Table 2.4. Proximity network measures during fruit and invertebrate feeding and foraging bouts.

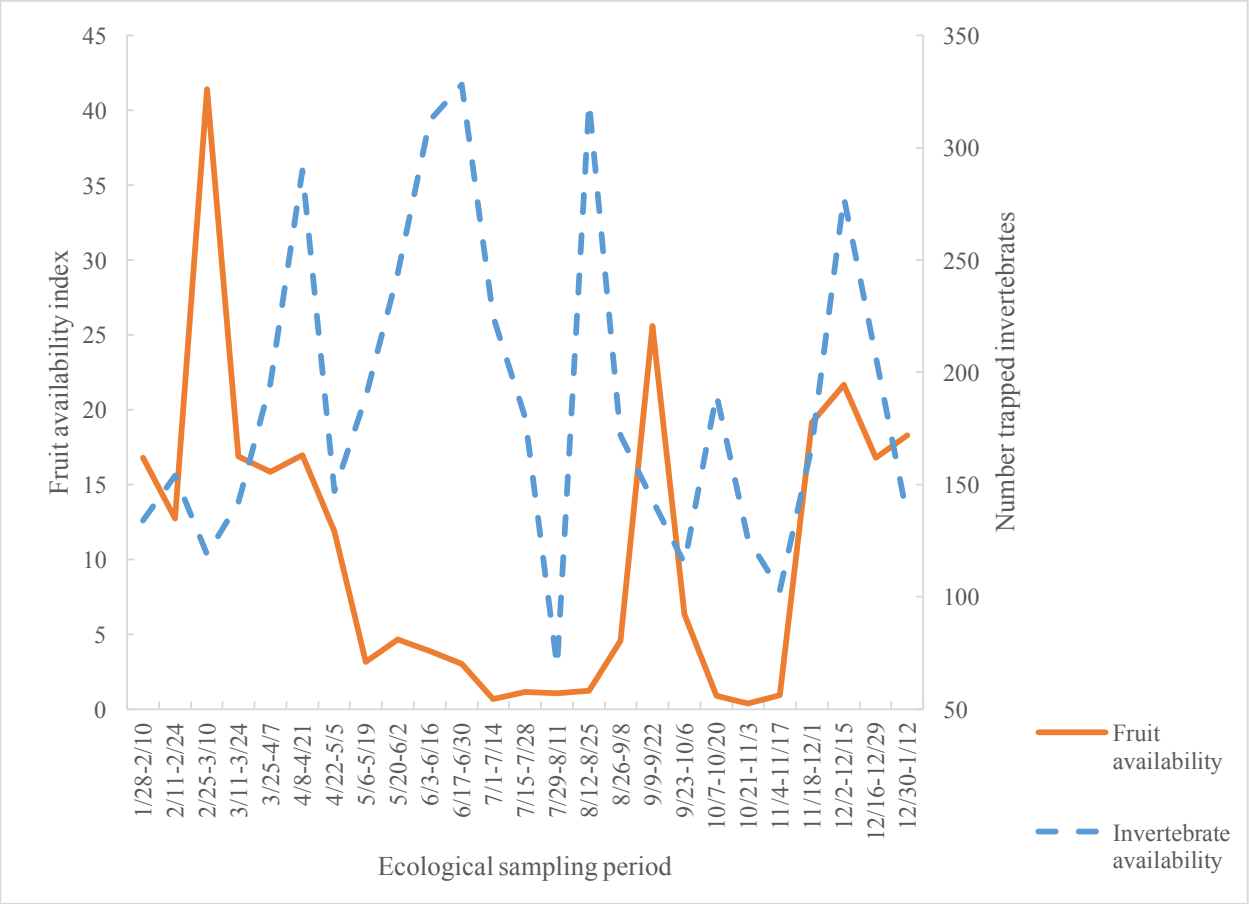

Figure 2.1. Fruit and invertebrate availability at La Suerte Biological Field Station from January 2013 through January 2014.

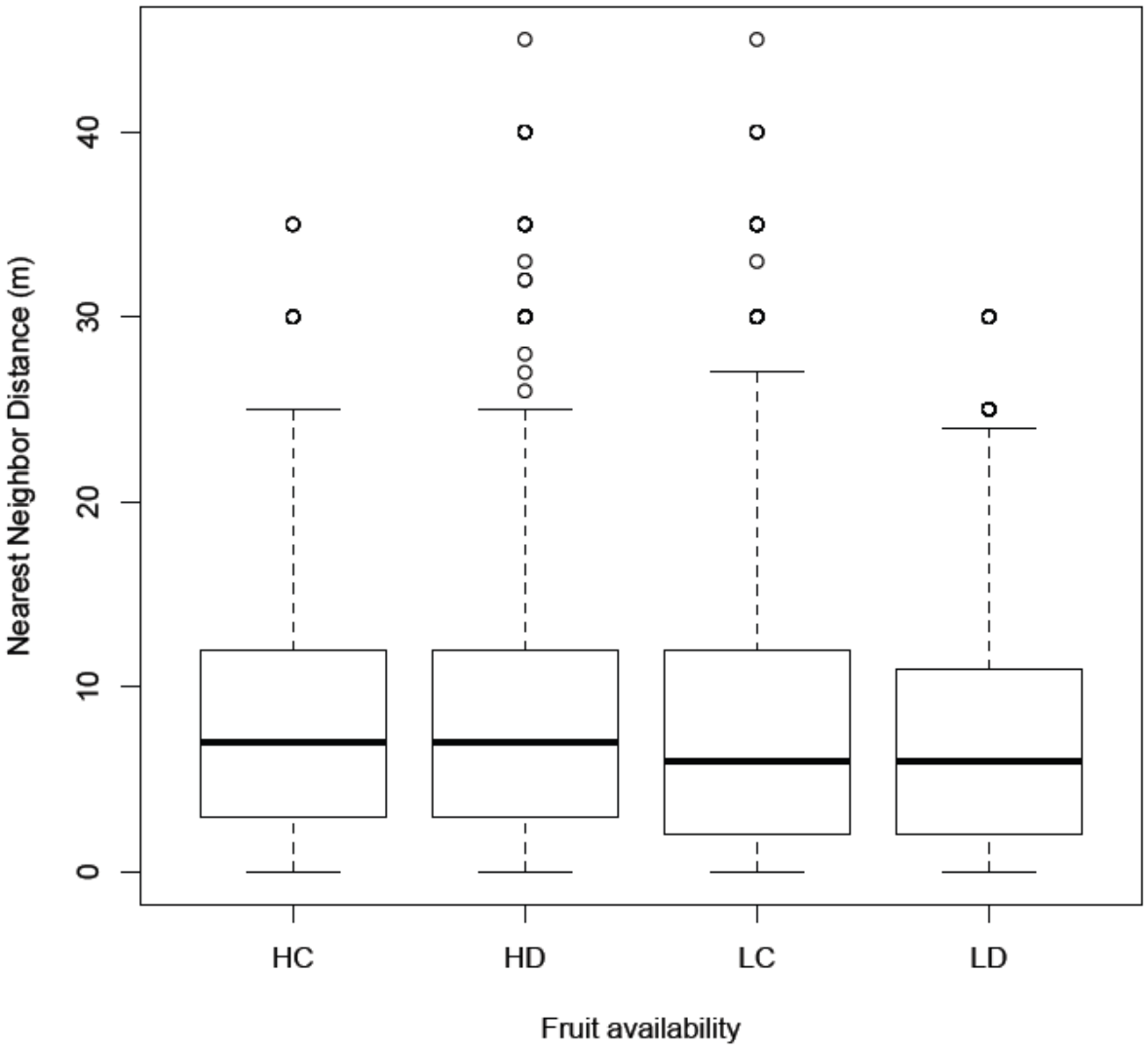

Figure 2.2. Average nearest neighbor distances during periods of high and low fruit abundance (H=high, L=low) and high and low patch density (C=clumped, D=dispersed).

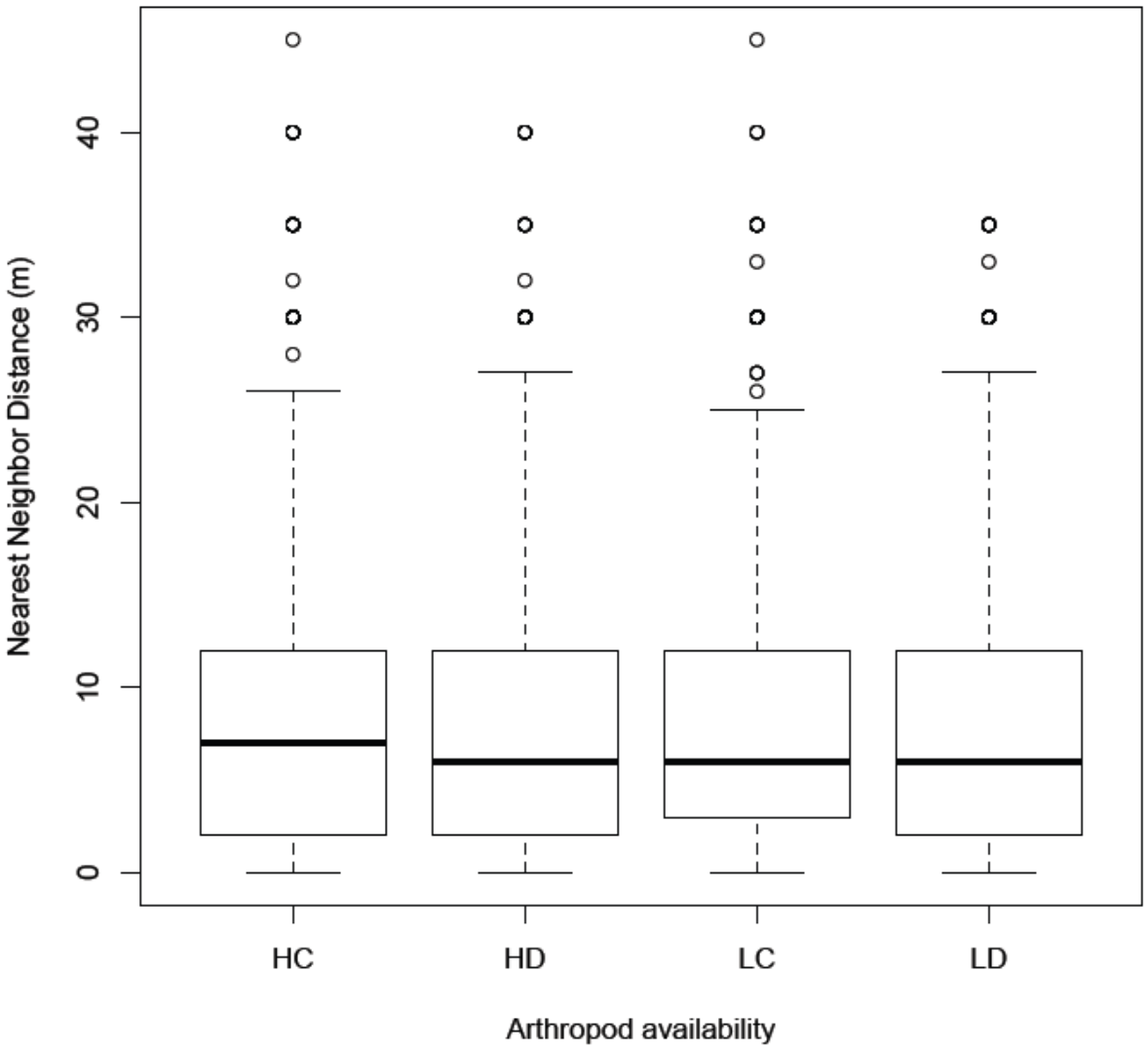

Figure 2.3. Average nearest neighbor distances during periods of high and low invertebrate abundance (H=high, L=low) and high and low patch density (C=clumped, D=dispersed).

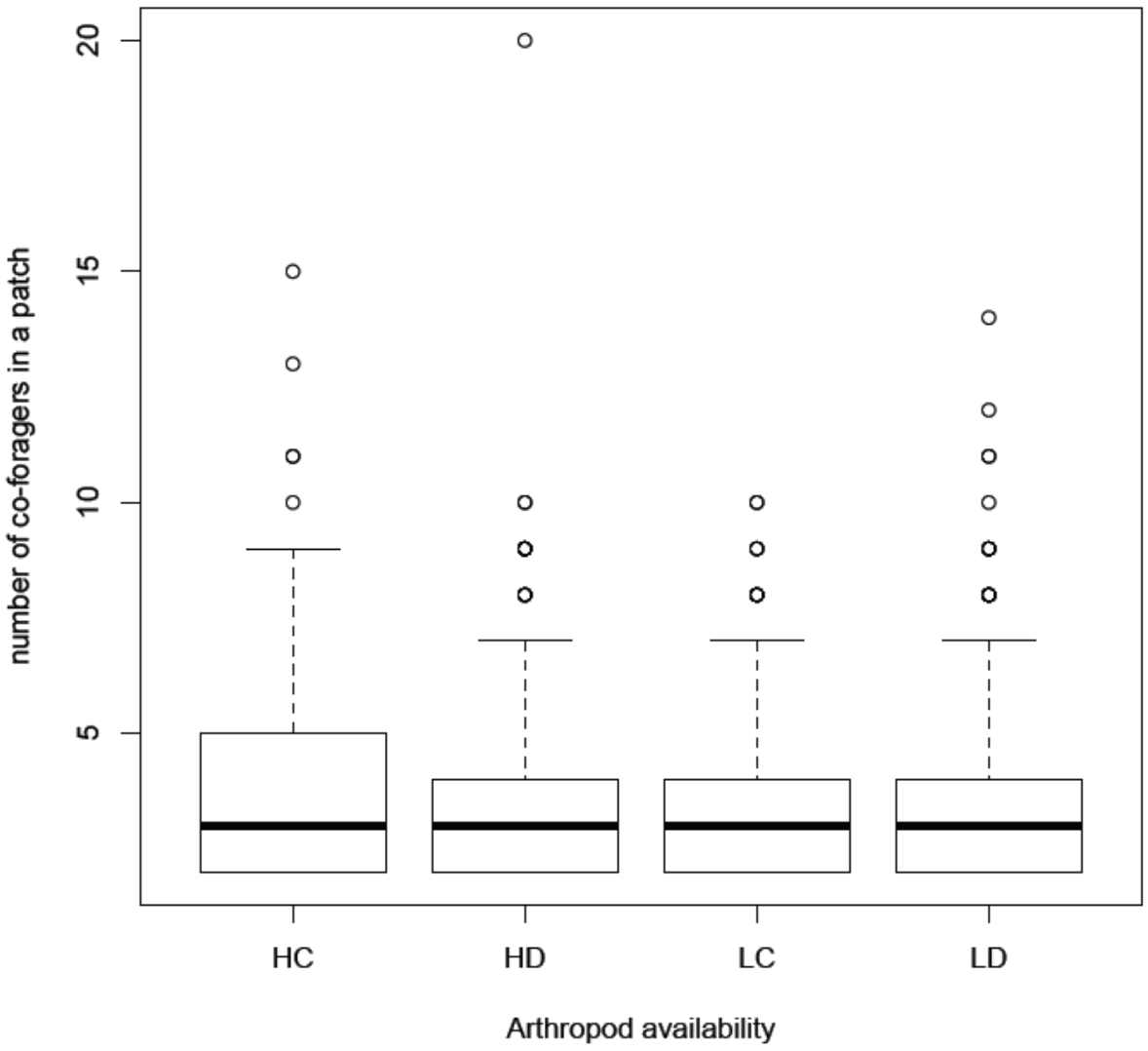

Figure 2.4. Average number of individuals co-foraging in a patch during periods of high and low invertebrate abundance (H=high, L=low) and high and low patch density (C=clumped, D=dispersed).

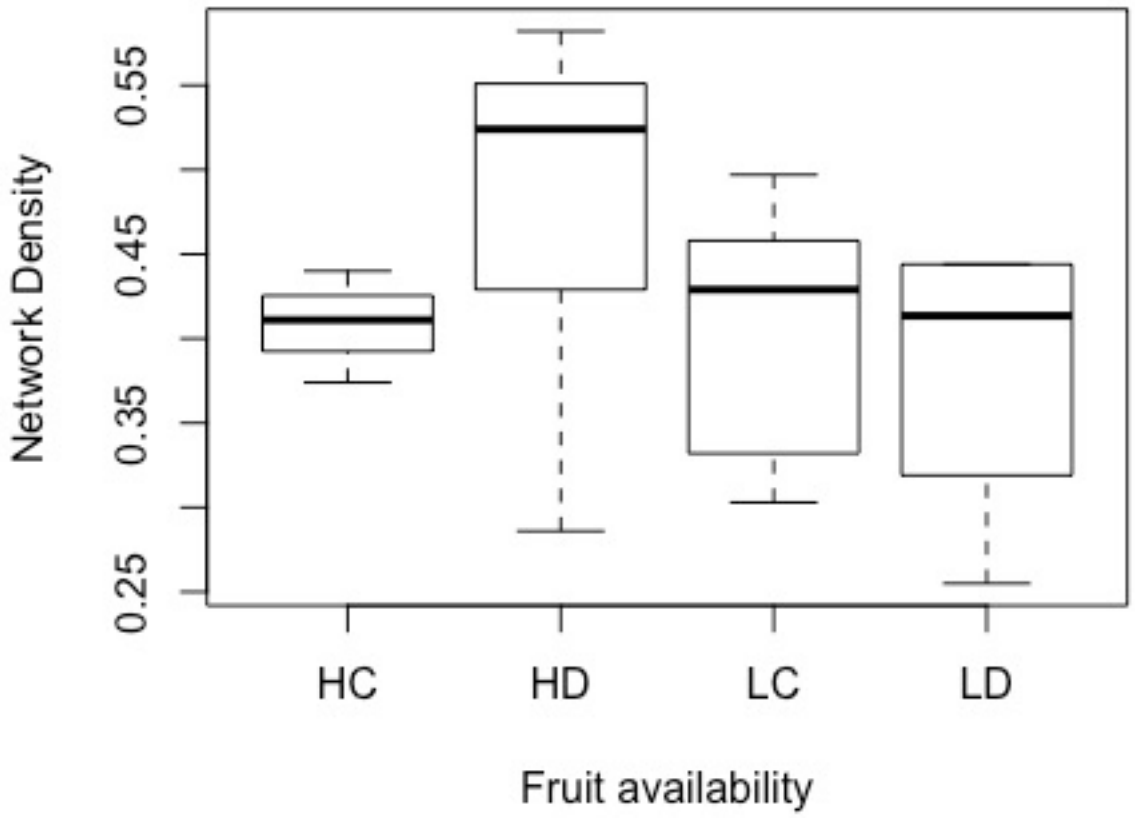

Figure 2.5. Average network density during foraging bouts in periods of high and low fruit abundance (H=high, L=low) and high and low fruit patch density (C=clumped, D=dispersed).

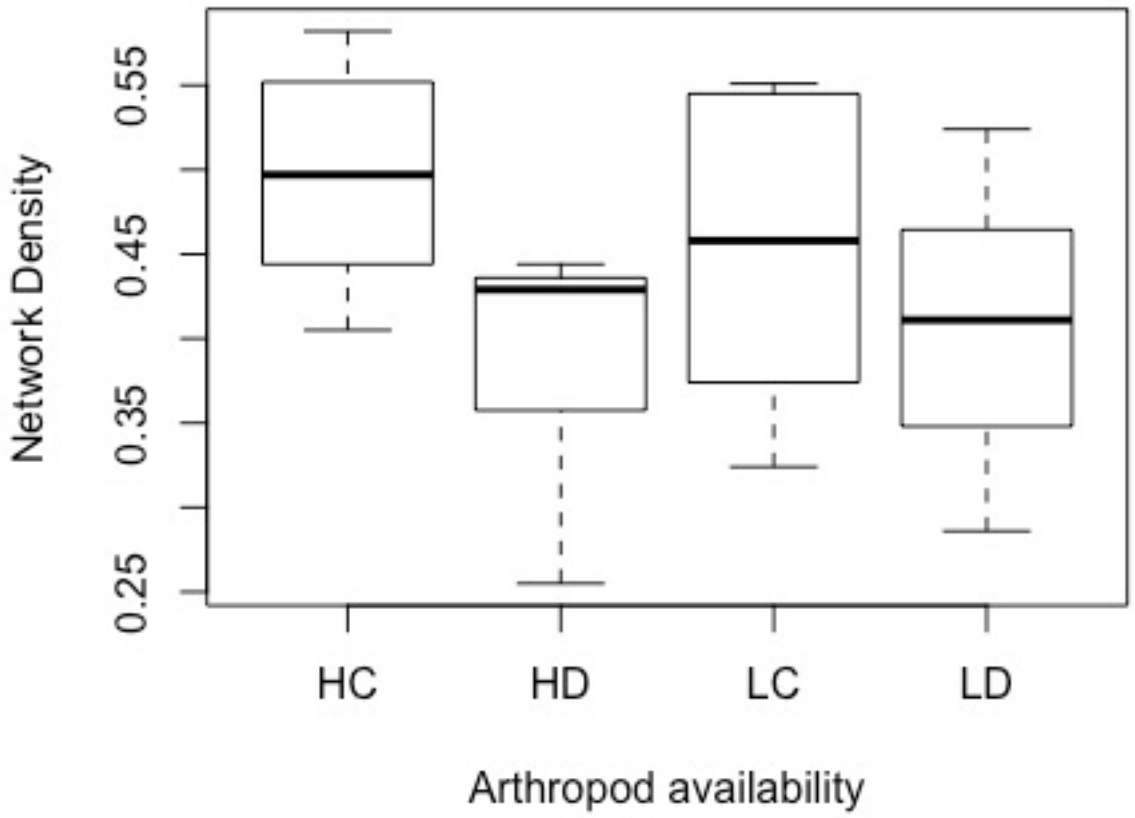

Figure 2.6. Average network density during foraging bouts in periods of high and low invertebrate abundance (H=high, L=low) and high and low invertebrate patch density (C=clumped, D=dispersed).

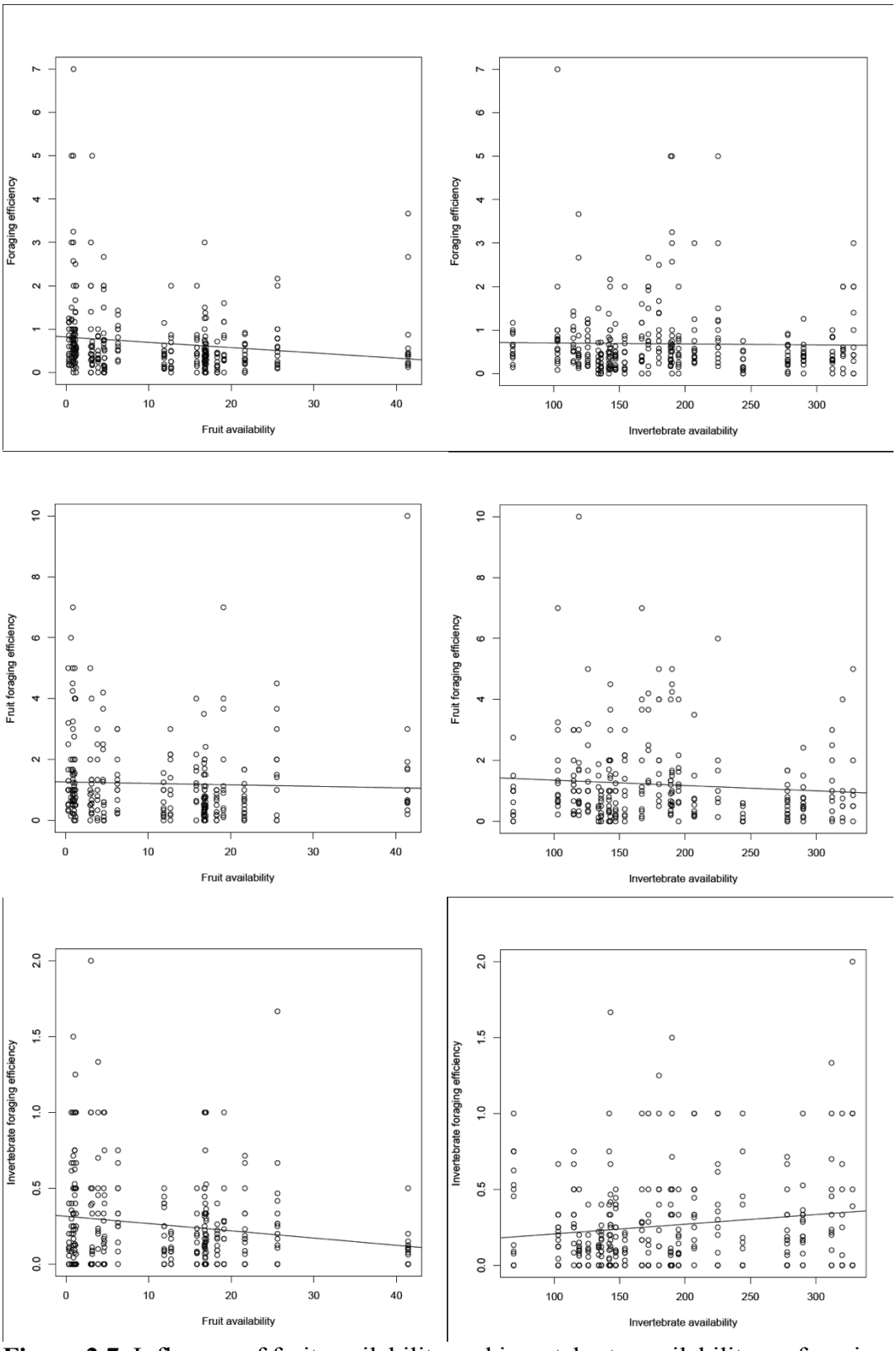

Figure 2.7. Influence of fruit availability and invertebrate availability on foraging efficiency during all feeding and foraging bouts, foraging efficiency during fruit feeding and foraging bouts, and foraging efficiency during invertebrate feeding and foraging bouts.

CHAPTER 3

INTEGRATING FEEDING BEHAVIOR, ECOLOGICAL DATA, AND DNA BARCODING TO IDENTIFY DEVELOPMENTAL DIFFERENCES IN INVERTEBRATE FORAGING STRATEGIES IN WILD WHITE-FACED CAPUCHINS (*CEBUS CAPUCINUS*)

Abstract

Invertebrate foraging strategies in nonhuman primates often require complex extractive foraging or prey detection techniques. As these skills take time to master, juveniles may have reduced foraging efficiency or concentrate their foraging efforts on easier to acquire prey than adults. We use DNA barcoding, behavioral observations, and ecological data to assess age-based differences in invertebrate prey foraging strategies in a group of white-faced capuchins (*Cebus capucinus*) in northeastern Costa Rica. Invertebrate availability was monitored using canopy traps and sweep netting. Fecal samples were collected from adult female, adult male, and juvenile white-faced capuchins (n=225). *COI* mtDNA sequences were compared to known sequences in GenBank and the Barcode of Life Database. Frequencies of Lepidoptera and Hymenoptera consumption were higher in juveniles than adults. A significantly smaller proportion of juvenile fecal samples contained Gryllidae and Cercopidae sequences, compared with adults (0% and 4.2% vs. 4.6% and 12.5%), and a significantly larger proportion contained Tenthredinidae, Culicidae, and Crambidae (5.6%, 9.7%, and 5.6% vs. 1.3%, 0.7%, and 1.3%). Juveniles spent significantly more time feeding and foraging than adults, and focused their foraging efforts on prey that require different skills to capture or extract. Arthropod availability was not correlated with foraging efficiency, and the rate of consumption of specific Orders of invertebrates was not correlated with the availability of those same taxa. Our data support the

hypothesis that juveniles do not have access to the same invertebrate prey as adults do, potentially due to differences in extractive foraging abilities.

Introduction

In nonhuman primates, animal prey foraging strategies involve complex searching and detection skills and extractive foraging techniques principally acquired during the juvenile period (Joffe, 1997; Ross and Jones, 1999; Johnson and Bock, 2004). In species such as chacma baboons and brown capuchins juvenile foraging strategies include focusing on more easily acquired foods or lowered foraging efficiency compared to adults (Ross and Jones, 1999; Johnson and Bock, 2004; Gunst et al., 2010). The needing-to-learn hypothesis posits that the extended juvenile period in primates evolved in part to allow individuals the time to learn the complex social and foraging skills necessary for later reproductive success (Joffe, 1997; Ross and Jones, 1999; Johnson and Bock, 2004). Arthropods are frequently considered a readily digestible source of proteins and lipids for primates, and individuals with higher energetic requirements, such as juveniles and pregnant and lactating females, may rely more heavily on invertebrates than adult males (Janson and Boinski, 1992; McGrew, 2001; McCabe and Fedigan, 2007). Embedded invertebrates, which include termites, beetles, true bugs, and some species of caterpillars, however, are not readily available to foragers, especially juveniles, as individuals must develop the necessary strength and motor skills to extract and process arthropods, but also must acquire the necessary cognitive abilities and knowledge required to search for, detect, and acquire concealed prey (Ross and Jones, 1999; Johnson and Bock, 2004; Gunst et al., 2010). For example in the case of wild chimpanzees (*Pan troglodytes*), juveniles do not acquire all of the necessary skills or the ability to link the tool-building and foraging tasks in the correct order for

fishing from termite mounds to result in acquiring any termites until 5.5 years of age (Lonsdorf, 2005). Lonsdorf (2005) did not report the age at which juveniles acquire adult-like proficiency in termite fishing, however. In contrast squirrel monkeys (*Saimiri sciureus*) juvenile acquire animal prey items at the same rate as adults or appear to be using the same foraging strategies by eight months of age, but focus their efforts on different substrates (dead leaves and palm foliage) or invertebrates found on the surface of leaves (Stone, 2006, 2007b). While chimpanzees slowly learn different components of a complex, multi-step foraging technique over a longer portion of their juvenile period, juvenile squirrel monkeys appear to achieve adult-like foraging competence relatively early (Lonsdorf, 2005; Stone, 2006, 2007b). It is unclear, however, in the case of squirrel monkeys, whether juveniles are feeding on the same invertebrates as adults (Stone, 2006, 2007b). In studies of many wild populations of nonhuman primates, 24-95% of invertebrate prey captures are reported as unknown (Terborgh, 1983; Peres, 1993; Nekaris and Rasmussen, 2003; Nadjafzadeh and Heymann, 2008; Urbani, 2009; Bryer et al., 2015), and, without specific data on the taxa of prey being consumed, it is difficult to conclude whether or not juveniles have similar predation rates on specific taxa of invertebrates when compared with adults.

In this study we use DNA barcoding, behavioral observations, and ecological data to assess age-based differences in invertebrate prey foraging strategies in wild white-faced capuchins (*Cebus capucinus*). DNA barcoding has been used successfully to identify invertebrate prey (Pickett et al., 2012; Hamad et al., 2014; Mallott et al., 2015), vertebrate prey (Hofreiter et al., 2010), and plant taxa (Bradley et al., 2007) in the diet of wild primates, as well as in bats, seals, and large cats (Deagle et al., 2005; Deagle and Tollit, 2006; Pons, 2006; Clare et al., 2009, 2011, 2014; Bohmann et al., 2011; Zeale et al., 2011; Shehzad et al., 2012; Sedlock et al., 2014). DNA barcoding uses short segments (100-300bp) of highly variable mitochondrial DNA to

identify species present in environmental samples, and can be used to noninvasively monitor diet in animals. In nonhuman primates, this technique can be combined with behavioral observation to examine in more detail the specific taxa of invertebrates or vertebrates present in the diet. This integrative approach allows us to develop a more complete understanding of capuchin invertebrate foraging behavior.

Capuchins are unusual among non-ape primates in that they are characterized by a large brain volume to body size ratio (72.95cc/3.29kg, n=23; Isler *et al.* 2008); exploit embedded or concealed prey (wood boring beetles, true bug larvae, some species of caterpillars; Janson & Boinski 1992; Panger *et al.* 2002; Gunst *et al.* 2010); and actively hunt vertebrate prey (coati pups, squirrels, frogs, lizards, bird eggs; Rose 1997). Capuchins have an unusually long juvenile period compared with primates of similar size. Female age at first birth ranges from 5-7 years of age in wild white-faced capuchin populations (Fedigan and Rose, 1995; Perry et al., 2008). Male white-faced capuchins in wild populations have first been reported to sire offspring at 7.8 years of age (Muniz et al., 2010) and reach adult size at 10 years of age (Jack and Fedigan, 2004). White-faced capuchin monkeys exploit a wide range of food types, devoting an average of 44.4% of feeding and foraging time to fruit (range=15-83.3%), 38.0% to invertebrates (range=0-68.3%), and 1.2% to vertebrates (range=0-4.2%) (Buckley, 1983; Rose, 1994; Baker, 1998; Urbani, 2009; McKinney, 2010).

Several authors have found that juvenile capuchins use different, and sometimes less efficient, foraging strategies than adults. For example, Gunst et al. (2008 and 2010) in a study of wild *Sapajus apella* found that, when foraging for larvae that must be extracted from bamboo, the number of captures per hour (0 larvae per hour for infants; 1.6 larvae per hour for small juveniles, 4.5 larvae per hour for large juveniles, and 6.5 larvae per hour for subadults) and the

use of reliable detection techniques (tap scan: 13.2±12.9 times per hour for juveniles, 50.3±31.0 times per hour for adults; visual inspect: 23.1±11.2% for juveniles, 56.0±13.5% for adults) increased with age. The percentage of time spent using rotten bamboo stalks as a foraging substrate for larvae decreased with age (4.6±4.5% of time in young juveniles, 0.9±1.0% in older juveniles, 0.1±0.1 in subadults, 0.1±0.2 in adults). These results suggest that differences between adult and juvenile body size, dental development, and extractive foraging ability decrease juvenile foraging success relative to that of adults (Gunst et al., 2008, 2010). Similarly, in a study of *Sapajus nigritus*, Agostini and Visalberghi (2005) found that the percentage of time spent foraging for invertebrates in woody microhabitats (e.g. tree trunks and branches) decreased significantly as age increased (young juvenile males: 41%, old juvenile males: 40%, adult males: 36%, young juvenile females: 34%, old juvenile females: 28%, adult females: 27%), suggesting that juveniles were targeting different foraging microhabitats than adults. Both older and younger juveniles were significantly less efficient than adults when foraging for animal prey, measured as the proportion of successful searches over the total searches attempted (Agostini and Visalberghi, 2005). In *Cebus olivaceus*, Fragaszy and Boinski (1995) juveniles were less efficient than adults of the same sex when foraging for plant foods (adult males = 77%; adult females = 47%; juvenile males = 51%; juveniles females = 32%). These authors argued that differences in foraging success was the result of both differences in body size and differences in knowledge of the location, availability, and capture methods for specific plant foods (Fragaszy and Boinski, 1995). Taken together, these data suggest that in some species, during prey foraging juveniles forage less efficiently than adults. In these species, juveniles also focus their foraging efforts on different microhabitats and may have access to different taxa of prey than adults. In contrast, studies of wild white-faced capuchins report limited evidence for age-based differences

in foraging behavior. In a study of white-faced capuchins, Bezanson (2009) found that adult and juveniles exhibited similar foraging efficiency across all food types (measured as a ratio of feeding to foraging time) by six months of age. However, in another study of white-faced capuchins, MacKinnon (1995) found that infants and young juveniles (less than 3 years of age) had higher rates of "grab and miss" per hour compared with adults when foraging for invertebrates (infants = 0.29-0.31; young juveniles = 0.03-0.11; old juveniles = 0.04-0.23; adults = 0.01-0.09).

Based on the needing-to-learn hypothesis, we test the following predictions about age-based differences in white-faced capuchin invertebrate foraging strategies. **1.** Assuming juveniles have not had sufficient time to learn adequate prey detection techniques and extractive foraging skills, juveniles will have decreased foraging efficiency (measured as a ratio of feeding to foraging time) when exploiting invertebrate prey relative to adults in order to meet their energetic needs; **2.** If juveniles are less efficient foragers for concealed or embedded prey (defined as invertebrates found in leaf rolls, embedded in bark and dead wood, or found inside of trees cavities) than adults, then fewer juvenile fecal samples are expected to contain sequences from these prey item Families compared with the percentage of fecal samples from adults and juvenile combined in which a given Family of invertebrates was found; **3.** Assuming juveniles do not have fully developed prey capture abilities when compared with adults, juveniles will consume highly mobile or flying invertebrate prey less frequently than expected based on the percentage of fecal samples collected from juveniles containing a Family of invertebrates; and **4.** If juveniles do not have access to the same diversity of arthropods as adults due to differences in foraging abilities, the frequency of occurrence of taxa in juvenile feces will be more highly correlated with the availability of that taxa when compared with adults.

Methods

Study site and population description

Research was conducted from January 2013 through January 2014 at La Suerte Biological Field Station (LSBFS) in northeastern Costa Rica (10.445N, 83.784W). LSBFS contains approximately 300 ha of aseasonal wet, lowland tropical forest, including 20 ha of primary forest, 150 ha of advanced secondary forest, and 130 ha of early secondary growth and regenerating pasture (Garber and Paciulli, 1997). Much of the early secondary growth and regenerating cattle pasture are part of a managed reforestation project currently underway at this private reserve (Garber et al., 2010). Yearly rainfall in this region of Costa Rica averages 3962 mm, and rainfall during the study period was 3116 mm (Garber and Rehg, 1999; Urbani, 2009). Four groups of *C. capucinus* are present, one of which is habituated. The group of habituated, individually recognizable *C. capucinus* that were used for the study contained 21-22 individuals (4 adult males, 5 adult females, 8-9 juvenile males, 2-3 juvenile females, and 0-2 infants) during the study period. Infants were defined as individuals that were dependent on their mother. Juveniles were defined as independent individuals 1-5 years of age, with age being determined by both forehead coloration and size relative to known adult individuals. These individuals were habituated to all day follows during a pilot study conducted in the summer of 2011. All data collection methods were approved by the University of Illinois IACUC, La Suerte Biological Field Station, MINAET, SINAC, and CONAGEBIO.

Observational data collection

Dawn-to-dusk 1-hour instantaneous focal animal samples (2-min interval) of individually recognizable adult males, adult females, juvenile males, and juvenile females were conducted 5 days a week (20 days/month) for 12 months. Juveniles were defined as independent individuals 1-5 years of age, with age being determined by both forehead coloration and size relative to known adult individuals. These individuals were habituated to all day follows during a pilot study conducted in the summer of 2011. In total, 841 hours of behavioral data during 1341 hours of observation on 237 days were collected. Behavioral data collection was biased towards the morning, as poor visibility due to rain, canopy height, or dense vegetation often made it difficult to stay with the group for an entire 12-hour day (66.4% of quantitative data was collected between 0500-1100). A slightly larger percentage of data were collected from adults in the morning (0500-1100) (67.8% of all adult behavioral observations) compared with the percentage of data collected from juveniles in the morning (60.7% of all juvenile behavioral observations). As such, we may be underreporting juvenile feeding and foraging behavior. Not all juveniles were individually recognizable during the early months of the study, so some juvenile focal follows were of individuals of unknown identity and sex. No two individuals of the same age- and sex-class were followed sequentially, and we attempted focal follows of all individuals in the group before following the same individual a second time during the day.

Information on activity budget (feeding, foraging, traveling, resting, social, other), diet (ripe fruit, unripe fruit, invertebrates, vertebrates, flowers, leaves, seeds, other). Invertebrate foraging included searching for invertebrates (turning over leaves, riffling through dead vines and other plant debris), manipulating substrates where arthropods might be found (ripping apart branches, removing bark from trees, manually exploring holes in trees, tearing apart termite nests, knocking wasp and bees nests out of trees), and processing invertebrates prior to

consuming them (removing exoskeletons, hairs and spines from caterpillars, legs, and/or wings). Data on social interactions (including affiliative, aggressive, and submissive behaviors) was also collected. When possible, consumed food items were identified to species (for fruit) or Order (for invertebrates).

Fecal sample collection

Between January 2013 and January 2014, 225 fecal samples were collected from adult female (n=64), adult male (n=88), juvenile females (n=15), juvenile males (n=47), and juveniles of unknown sex (n=11). The number of samples collected per two-week period per age/sex class ranged from 1-6. An effort was made to collect fecal samples from all age/sex classes evenly across the study period, so that at least one sample from an adult female, an adult male, and a juvenile male was collected in each two-week period. As there were few juvenile females in the study group, we were only able to collect samples from juvenile females in 12 of the 25 two-week periods. Fecal sample collection occurred throughout the day, but collection was biased towards the morning (145 morning samples vs. 80 afternoon samples). Samples were collected in a manner to minimize contamination from exogenous sources, with care taken to avoid collecting material in contact with the forest floor or leaf litter. Samples were stored in 90% ethanol at -20°C prior to being shipped to the Molecular Anthropology Laboratory at the University of Illinois at Urbana-Champaign.

Invertebrate availability data collection

Arthropod availability was assessed by bi-monthly collections using 10 Composite Insect Traps (Russo et al., 2011), measuring 0.5x1.5 m, placed at randomly chosen geographic

coordinates within a 20.2 ha area of the study group's home range, hoisted on a pulley system to 5-15 m above the ground. Each trap was consistently located at the same height, but the overall height of the canopy in some trap locations limited how high we could hang the traps. Canopy traps were hung for 72 hours every two weeks. Sweep netting (0.5 m diameter net, height of 1.5 m) was conducted for 1 minute at trap locations between 0700 and 1300 every two weeks, coinciding with days when arthropods were collected from the canopy traps. On average, we swept 30 times per minute. We avoided sweep netting during heavy rain, but it was not always possible to avoid collection during light rain and rarely possible to avoid sweeping when vegetation was wet. The use of both sweep netting and Composite Insect Traps sampled multiple taxa of both flying and crawling arthropods. Arthropods from each trap were counted and identified to Family using classification keys and reference books in the library at La Suerte Biological Field Station. Species richness and a Shannon diversity index were calculated for each trapping period.

DNA extraction

DNA was extracted from 225 fecal samples using a QIAamp DNA Stool Mini Kit following the provided protocol for "Isolation of DNA from Stool for Human DNA Analysis." Negative extraction controls were performed. Samples were homogenized using a vortexer prior to extraction. The following modifications from Pickett et al. (2012) were used. Samples were mixed with Buffer ASL and then incubated for 1 hour at room temperature. Next, Buffer AL was added to the samples, which were then incubated for an additional 20 minutes at 70°C prior to elution. During the final elution step, Buffer AE was pre-warmed to 70°C, added to the samples, and incubated for 20 minutes at room temperature before spinning down the columns.

PCR amplification

Two overlapping ~300bp fragments within the *COI* mitochondrial gene were amplified using universal arthropod primers (Mallott et al., 2015). For 56 of the 225 samples, PCR reactions were carried out in a total volume of 30 µl, consisting of 4 µl DNA, 2.5 µl 10X HiFi platinum Taq buffer (Roche), 1 µl 10 mM dNTPs, 1 µl 50 mM $MgSO_4$, 1 µl 20 µM forward primer, 1 µl 20 µM reverse primer, 0.2 µl (1 Unit) platinum HiFi Taq polymerase (Roche), and 18.3 µl molecular grade H_2O. Cycling was performed using the following program: 5 min at 94°C, followed by 40 cycles of 30 sec at 94°C, 60 sec at 50°C (for primer pair 1) or 55.5°C (primer pair 2), 30 sec at 72°C, followed by 5 min at 72°C and then held at 4°C indefinitely. PCR products were digested with *BsrFI*, which contains a restriction site in primate *COI* genes, but not insect *COI* genes. Five units of the restriction enzyme *BsrFI* was added to 20 µl of PCR product and incubated at 37°C for 2 hours. Samples were then exposed to electrophoresis on a 2% agarose E-Gel (Life Technologies), and the desired band was excised from the gel. Insect *COI* fragments were extracted from the gel using an Omega gel extraction kit based on the manufacturer's provided protocol. Gel extracts were amplified with PCR conditions and program described above (with 30 cycles instead of 40) using HPLC-purified fusion primers. Fusion primers consisted of the original insect primers, plus an adaptor sequence necessary for Illumina sequencing added to the 5' end of both the forward and reverse primers. A unique 8 bp barcode tag also was added to the forward and reverse primer between the adaptor sequence and the primer sequence to identify individual samples. Amplicons were AMPure-bead purified, DNA concentration was quantified using a Qubit assay, and samples were diluted to provide an equal

concentration in the pool to be sequenced. The pool was sequenced on the Illumina MiSeq platform using a Nano kit.

The remaining 169 samples were amplified by PCR using primer pair 2 (see above) on a Fluidigm Access Array at the Roy J. Carver Biotechnology Center at UIUC. A Fluidigm Access Array allows for the amplification of a sample with up to 48 different primer pairs simultaneously, in addition to combining the amplification of the target region and barcoding of the amplicon in the same reaction, minimizing contamination. Prior to amplification, all samples were quantitated on a Qubit (Life Technologies) using the High Sensitivity DNA kit and then diluted to a 2 ng/µl concentration. A mastermix for amplification was prepared using the Roche High Fidelity Fast Start Kit and 20x Access Array loading reagent according to Fluidigm protocols. For each sample, the following reagents were combined: 0.5 µl 10X FastStart Reaction Buffer without $MgCl_2$, 0.9 µl 25 mM $MgCl_2$, 0.25 µl DMSO, 0.1 µl 10 mM PCR grade Nucleotide Mix, 0.05 µl 5 units/µl FastStart High Fidelity Enzyme Blend, 0.25 µl 20X Access Array Loading Reagent, 0.95 µl molecular grade H_2O. Mastermix was aliquoted to 48 wells of a PCR plate. To each well, 1 µl DNA sample and 1 µl Fluidigm Illumina linkers with unique barcodes were added. In a separate plate, primer pairs were prepared and aliquoted. 20X primer solutions were prepared by adding 2 µl of each forward and reverse primer, 5 µl of Access Array Loading Reagent, and molecular grade H_2O to a final volume of 100 µl. 4 µl of sample was loaded in the sample inlets and 4 µl of primer solution was loaded in the primer inlets of a previously primed Fluidigm 48.48 Access Array IFC. The IFC was placed in an AX controller (Fluidigm Corp.) for microfluidic loading of all primer/sample combinations. Following the loading stage, the IFC plate was loaded on the Fluidigm Biomark HD PCR machine and samples were amplified using the following Access Array cycling program without imaging: 2 min at

50°C, followed by 20 min at 70°C, followed by 10 min at 95°C, followed by 10 cycles of 15 sec at 95°C, 30 sec at 60°C, 1 min at 72°C, followed by 2 cycles of 15 sec at 95°C, 30 sec at 80°C, 30 sec at 60°C, 1 min at 72°C, followed by 8 cycles of 15 sec at 95°C, 30 sec at 60°C, 1 min at 72°C, followed by 2 cycles of 15 sec at 95°C, 30 sec at 80°C, 30 sec at 60°C, 1 min at 72°C, followed by 8 cycles of 15 sec at 95°C, 30 sec at 60°C, 1 min at 72°C, followed by 5 cycles of 15 sec at 95°C, 30 sec at 80°C, 30 sec at 60°C, 1 min at 72°C. Following amplification, 2 µl of Fluidigm Harvest Buffer was loaded in the sample inlets and loaded on the AX controller for harvesting PCR products. Harvested product was then transferred to a new 96 well plate, quantitated on a Qubit, and stored at -20°C. All samples were run on a Fragment Analyzer (Advanced Analytics, Ames, IA) and amplicon regions and expected sizes were confirmed. Samples were then pooled based on product concentration, and were gel purified from a 2% agarose E-gel using a Qiagen gel extraction kit. Individually barcoded samples were then sequenced on the Illumina MiSeq platform at the Roy J. Carver Biotechnology Center at UIUC.

Sequence processing

Sequencing yielded an average of 6,435 raw reads per sample. Raw sequence reads were demultiplexed and trimmed in QIIME (Caporaso et al., 2010). Reads were de-replicated, clustered at a 1% sequence divergence threshold, and chimeric sequences were detected denovo and removed in USEARCH (Edgar, 2010; Edgar et al., 2011). Sequence reads were then clustered into operational taxonomic units (OTUs) using a 3% sequence divergence criteria (see Mallott *et al.*, 2015 for a discussion of choice of sequence divergence criteria). OTUs were then compared to the GenBank nt nucleotide sequence database and the Barcode of Life Database

(www.boldsystems.org) using BLAST (ncbi.nlm.nih.gov). OTUs were then assigned to taxa in MEGAN5 using the standard settings (Huson et al., 2011).

Statistics

A linear mixed effects model was used to examine the effects of food availability, age, and sex on minutes per hour spent feeding and foraging, percent of total feeding and foraging time spent searching for and consuming invertebrates, invertebrate feeding rates, and foraging efficiency. Invertebrate availability and age were included as fixed effects, and individual was included as a random effect to control for pseudoreplication. Fruit availability and sex were also controlled for in the model. Linear mixed effects models were also used to examine the effect of age on frequency of consumption of Orders of invertebrates, controlling for both sex and individual. The *nlme* package in R (r-project.org) was used to run all linear models. A chi-squared goodness-of-fit test was used to test the expected versus observed number of samples containing a given Family of invertebrates for adults and juveniles based on the percentage of total samples collected from adults and juveniles and the number of samples in which a given Family of invertebrates was found ($expected\ samples\ for\ adults = (number\ of\ samples\ containing\ each\ Family) * (\frac{number\ of\ samples\ from\ adults}{total\ fecal\ samples})$. Due to the small sample size, significance was tested at p=0.1 for chi-square goodness-of-fit tests. Pearson's product-moment correlation was used to test for the significance of the relationships between invertebrate availability and the frequency of invertebrate Orders found in the fecal samples.

Results

Overview of behavioral data

The group contained 21-22 individuals during the study period, including 4 adult males, 5 adult females, 8-9 juvenile males, 2-3 juvenile females, and 0-2 infants. The activity budget of the group during the study was 12.6% feeding, 26.3% foraging, 17.8% resting, 13.8% social, and 29.5% traveling (Table 3.1). During the study period, the group spent 47.8% of their feeding and foraging time on fruit, 1.1% on flowers, 0.6% on leaves, 45.9% on invertebrates, 0.04% on vertebrates, and 0.6% on other (Table 3.2). Average foraging efficiency on all food types was 0.5 minutes feeding per minute foraging, and average foraging efficiency for invertebrates was 0.2 minutes feeding per minute foraging. There was no significant effect of sex on minutes per hour spent feeding and foraging, percentage of feeding and foraging time devoted to invertebrates, fruit foraging efficiency, or foraging efficiency when all food types are combined (all $p>0.05$). Therefore, for these variables, males and females are combined in the following analyses of age-based differences. There was, however, a significant effect of sex on invertebrate foraging efficiency ($t=2.71$, $df=23$, $p=0.0124$). Adult males were more efficient than adult females (0.23 ± 0.11 vs. 0.16 ± 0.02) and juvenile males were more efficient than juvenile females (0.21 ± 0.11 vs. 0.18 ± 0.04). However, juvenile males did not differ significantly from either adult males or adult females. Therefore, in all analyses, adult females, adult males, juvenile females, and juvenile males are considered separately.

Does juvenile invertebrate foraging behavior differ from that of adults?

There was no significant effect of age class on fruit, invertebrate, or overall foraging efficiency or percent of feeding and foraging time spent on invertebrates among adult males, adult females, juvenile males, and juvenile females (all $p>0.05$). There was a significant effect of age on minutes per hour spent feeding and foraging on all food types and minutes per hour spent

feeding and foraging on invertebrates, with juveniles spending more time than adults feeding and foraging overall and specifically on invertebrates (t=2.88, df=23, p=0.0084; t=2.61, df=23, p=0.0158) (Figure 3.1 and Table 3.3). These data indicate that, while there is no difference in foraging efficiency, juveniles are spending more time feeding and foraging on invertebrates than adults. This does not support the first hypothesis that juveniles are less efficient foragers, even though they are spending more time feeding and foraging. Therefore, our results do not indicate that juveniles are less skilled foragers when compared with adults.

Are juveniles and adults consuming different taxa of invertebrates?

During the observational study, we were able to identify the Order and developmental stage of insect consumed 5.2% of the time for adult males, 1.3% for adult females, and 1.1% for juveniles. White-faced capuchins were observed eating 8 Orders of invertebrates: Hymenoptera (29.3% of identified captures), Orthoptera (15.2%), Hemiptera (14.1%), Isoptera (14.1%), Phasmatodea (14.1%), Lepidoptera (7.6%), Araneae (3.2%), and Blattodea (2.1%). Juveniles were observed consuming Araneae and Hymenoptera more frequently than adults (18.1% vs. 1.2% and 36.3% vs. 28.4% of observed captures), and Isoptera less frequently than adults (0% vs. 16.0%) (Table 3.4). Juveniles were never observed consuming Blattodea or Isoptera. Thus, overall we found that juveniles consumed easier to capture or extract Orders of invertebrates or Orders that are known to be consumed in larval form by capuchins more often than adults (81.81% of observed captures, n=11 vs. 66.67%, n=81).

The molecular analysis identified 29 Orders, 90 Families, 287 genera, and 190 species of arthropod in white-faced capuchin feces during the study period (Appendix C). Lepidoptera (45.6% of samples), Diptera (38.5%), Coleoptera (31.9%), Hymenoptera (27.0%), Hemiptera

(23.5%), Orthoptera (16.4%), Araneae (10.2%), Decapoda (8.8%), Blattodea (7.7%), and Mantodea (4.0%) were the most frequently consumed Orders of Arthropods (Figure 3.2). These values are based on the frequency of occurrence of each Order in the fecal samples. There was no effect of sex on frequency of consumption of any Order of invertebrates (all $p>0.05$). Juveniles consumed Lepidoptera and Hymenoptera significantly more frequently than adults ($t=2.20$, $df=18$, $p=0.0411$ and $t=2.19$, $df=18$, $p=0.0417$) (Table 3.5).

Do juveniles consume concealed, embedded, or highly mobile prey at the expected frequency?

Based on molecular data, a higher proportion of samples containing Crambidae (grass moths), Culicidae (mosquitos), Tenthredinidae (sawflies) were from juveniles compared with the overall proportion of juvenile samples ($\chi^2=3.29$, $p=0.070$, $\chi^2=11.29$, $p<0.001$; $\chi^2=3.29$, $p=0.070$), and a lower proportion of samples containing Gryllidae (crickets) and Cercopidae (spittlebugs) were from juveniles compared with the overall proportion of juvenile samples s ($\chi^2=3.33$, $p=0.068$; $\chi^2=3.47$, $p=0.062$) (Table 3.6). Juveniles are eating prey Families that are concealed while in larval form at higher than expected frequency (Tenthredinidae and Crambidae), contrary to expectations, and are eating other concealed taxa at the expected frequency. This should be interpreted conservatively, however, as molecular data cannot indicate whether or not prey was eaten in larval or adult form. Juveniles are not eating some Families of highly mobile prey (Gryllidae and Cercopidae) at the expected frequency, however, other taxa of highly mobile prey are being eaten at the expected frequency (Acrididae and Salticidae).

Are adult and juvenile prey foraging strategies influenced differently by changes in invertebrate availability?

Total arthropod abundance in traps and sweep samples was significantly negatively correlated with the frequency of Mantodea consumption in juveniles ($t=2.55$, $df=22$, $p=0.0181$, $r=-0.478$), but not in adults. Total arthropod abundance was not significantly correlated with the frequency of consumption of other Orders of invertebrates in juveniles (all $p>0.05$). Total arthropod abundance was not significantly correlated with the frequency of consumption of any Orders of invertebrates in adults (all $p>0.05$). The abundance of specific Orders of arthropods in the environment was not significantly correlated with the frequency of consumption of those same Orders of invertebrates in the diet of either adults or juveniles (all $p>0.05$). This suggests that white-faced capuchin invertebrate consumption is independent of the availability of those invertebrates in the environment.

Total time spent feeding and foraging was influenced significantly by total invertebrate availability in traps and sweep samples, decreasing with increasing invertebrate availability ($t=2.03$, $df=308$, $p=0.0436$) (Figure 3.3). The percentage of feeding and foraging time devoted to invertebrates was also influenced significantly by invertebrate availability, increasing as invertebrate availability increased ($t=2.06$, $df=308$, $p=0.0402$) (Figure 3.4). Foraging efficiency was not significantly influenced by invertebrate availability in adult males, adult females, juvenile males or juvenile females for any food type ($p>0.05$). Thus, adult and juvenile foraging behavior and prey choice was similarly unaffected by fluctuations in invertebrate prey availability, with the exception of Mantid consumption being tied to overall invertebrate availability in juveniles.

Discussion

This study tested the needing-to-learn hypothesis by examining whether juvenile white-faced capuchin invertebrate prey foraging strategies differ from those of adults. As the adult-like extractive foraging strategies and prey detection skills in capuchins likely take many years to fully develop, we expected to see differences in adult and juvenile invertebrate prey foraging behavior and differences in the arthropod prey to which adults and juveniles had access. Juveniles spent more time feeding and foraging than adults during the study period, both on all food types and specifically on invertebrates. Molecular sequences from Lepidoptera and Hymenoptera were found in a significantly greater proportion of juvenile fecal samples compared with those from adults. Additionally, there were large differences in the rates of Coleoptera, Hemiptera, and Diptera consumption between adults and juveniles; however, these differences were not statistically significant. Lepidoptera (caterpillars) and Hymenoptera (bees, wasps, ants) are invertebrate taxa that have been reported as being consumed in larval form by capuchins and were consumed at higher frequency by juveniles (61.3% and 44.0% of fecal samples, respectively) than adults (43.0% and 20.4%) in the present study (Lepidoptera: (Janson, 1985b; Robinson, 1986; Janson and Boinski, 1992; Rose, 1994; Galetti and Pedroni, 1994; Fragaszy and Boinski, 1995; Mackinnon, 1995; Agostini and Visalberghi, 2005; McCabe, 2005; MacKinnon, 2006; McKinney, 2010; Gunst et al., 2010; Melin, 2011; de Oliveira et al., 2014; Melin et al., 2014); and Hymenoptera: (Janson, 1985b; de Ruiter, 1986; Robinson, 1986; Janson and Boinski, 1992; Fragaszy and Boinski, 1995; Mackinnon, 1995; Panger et al., 2002; McCabe, 2005; Melin, 2011; Wheeler and Hammerschmidt, 2012; Lynch Alfaro et al., 2012; de Oliveira et al., 2014; Melin et al., 2014)), while Coleoptera (beetles) and Hemiptera (true bugs) are taxa that often require extractive foraging techniques and were consumed more frequently by adults (37.1% and 28.2% of fecal samples, respectively) than juveniles (26.0% and 15.3%). At the

Family level, prey taxa that are highly mobile in adult form (Gryllidae and Cercopidae) were eaten less frequently than expected by juveniles. Additionally, Cercopidae nymphs are frequently leaf miners, so may be difficult for monkeys to detect prior to the adult stage. These results are in line with previous studies of the development of invertebrate foraging behavior in capuchins that have suggested juveniles rely more heavily on larval forms of prey or surface invertebrates, and are less efficient than adults when foraging for invertebrates, especially when foraging for embedded or difficult to catch arthropods (Agostini and Visalberghi, 2005; MacKinnon, 2006; Gunst et al., 2008, 2010). However, in the present study, taxa that are frequently concealed in galls or bore into stems as larvae (Crambidae and Tenthredinidae) and would require advanced detection or extractive foraging skills, were eaten more frequently than expected by juveniles. Our results suggest that, while juvenile white-faced capuchins may be similar to adults in terms of foraging efficiency and may be imitating adult foraging behaviors at an early age, they are ingesting different Orders of invertebrates, indicating that juvenile capuchin dietary choices differ from those of adults, at least for some Orders of invertebrates. This may be due to differences in nutritional requirements, as juvenile capuchins need readily digestible sources of lipids and protein for growth and development, or may be due to the time required to fully develop the necessary motor skills to catch highly mobile prey or complex extractive foraging techniques required to access some invertebrate prey (Ross and Jones, 1999; Johnson and Bock, 2004; Gunst et al., 2010). Bezanson (2009) found that white-faced capuchins exhibit adult-like locomotor patterns by six months of age, making it unlikely that juvenile capuchins, age 12 months to five years, lack the motor skills to successfully capture mobile prey. While there are no data on postnatal brain growth in white-faced capuchins, brown capuchins (*Sapajus apella*) are reported to reach maximum total brain volume by 2.5 years of age (Phillips and Sherwood,

2008). As this nutritionally expensive period of rapid brain growth is over relatively early in the juvenile period, it is not clear by how much the nutritional requirements of juveniles would differ from those of adults in white-faced capuchins. Thus, based on evidence of similarities in foraging efficiency and observed invertebrate foraging behavior, and differences in invertebrate prey choice and the rate at which adults and juveniles consume highly mobile or embedded invertebrates, we feel our results indicate that differences juvenile and adult invertebrate foraging abilities does support the needing-to-learn hypothesis that the extended juvenile period is necessary in order to learn more complex foraging techniques. However, additional data from studies sites with juveniles of known age on how invertebrate foraging techniques and dietary choices change over the course of the juvenile period would give further clarity.

Additionally, we have gained insight into how invertebrate availability influences both invertebrate foraging behavior and dietary choices in white-faced capuchins. Even though both invertebrate availability are influencing foraging behavior by changing the amount of time spent feeding and foraging, arthropod availability is not influencing the frequency of consumption of invertebrate taxa. Specifically, white-faced capuchins devoted more time feeding and foraging for invertebrates when overall arthropod availability was higher. This is in contrast to a study of invertebrate foraging behavior in squirrel monkeys where individuals spent more time feeding and foraging for invertebrates during the dry season, even though insect biomass did not vary seasonally (Stone, 2007a). This difference may be attributed to the lack of seasonality in rainfall at LSBFS. Alternatively, it may be due to differences in the complexity of foraging strategies employed by capuchins when compared with those of squirrel monkeys, as squirrel monkeys do not perform the same destructive foraging techniques as capuchins and focus their invertebrate

foraging efforts on surface invertebrates or arthropods concealed in leaf rolls (Janson and Boinski, 1992).

Interestingly, the frequency with which white-faced capuchins consumed specific Orders of invertebrates based on molecular data is not influenced by the availability of those Orders in the environment. In the only other study of primate foraging behavior that both reported the availability and rate of consumption of specific Orders of invertebrates, changes in the frequency of consumption of invertebrate Orders by tarsiers was not directly related to changes in the availability of those Orders (Gursky, 2000). However, Gursky (2000) also reports that tarsiers did change their foraging strategies in response to fluctuations in the overall availability of invertebrates, principally by increasing their day range, decreasing their consumption of Orthopterans and Lepidopterans, and increasing their consumption of Coleopterans and Hymenopterans during the dry season. Gursky (2000) suggests in her study that differences between the response of tarsiers and New World monkeys to seasonal changes in invertebrate resource availability may be attributed to basic differences in flora and fauna in Southeast Asia and Latin America, or due to the diversity of invertebrate foraging strategies employed by New World monkeys. The complex and extractive invertebrate prey foraging techniques employed by capuchins may allow them to buffer variations in the availability of prey and to target specific taxa of invertebrates (Melin et al., 2014; Mosdossy et al., 2015), indicating that it is more likely that differences in foraging strategies, not flora and fauna, are driving differences in tarsier and capuchin responses to changes in invertebrate availability. By combining both behavioral and molecular data, the results of this study suggest that white-faced capuchins were able to consume taxa of invertebrates such as Hymenoptera at a fairly constant rate throughout the year.

The results of this study expand our knowledge of the diversity of invertebrate prey in white-faced capuchin diet. Of the 29 identified Orders, 20 have not been previously identified in white-faced capuchin diets. Compared with previous studies using DNA barcoding in New World primates, this study indicates that white-faced capuchins have a more diverse arthropod diet than other New World primates. For example, DNA barcoding identified 11 Orders, 15 Families, and 12 genera of arthropods in the diet of saddleback tamarins (*Saguinus weddelli*) (Mallott et al., 2015), and 3-15 arthropod taxa in the diets of squirrel monkeys (*Saimiri sciureus)*, white-fronted capuchins (*Cebus albifrons*), red woolly monkeys (*Lagothrix poeppigii*), equatorial sakis (*Pithecia aequatorialis*), red titis (*Callicebus discolor*), and spider monkeys (*Ateles belzebuth*) (Pickett et al., 2012). The substantially greater number of taxa found in our study is possibly due to the length of the study (12 months vs. 1 month in the other studies), the number of samples assayed (235 in our study vs 20 or less in the other studies), difference in sequencing methodology, and, in the case of Pickett et al. (2010), the difference in barcoding gene used, making it more likely that we identified both rarely eaten taxa as well as the diversity of taxa over the course of a year. However, a comparison may be made by calculating richness from 20 randomly selected samples per month from our study. Using the *rareNMtests* package in R (r-project.org), the estimated richness for 20 samples from a given month is 14.56 Orders, 30.30 Families, and 37.57 genera which is greater than the number found in saddleback tamarins. In comparison to the results from Pickett et al. (2012), the estimated richness in three white-faced capuchin samples from our study is 5.79 Orders and 6.62 Families, which is lower than that of *Saimiri*, but higher than that of the six other New World monkey genera sampled.

Caution must be employed when using DNA barcoding techniques due to primer biases, amplification stocasticity, and lack of knowledge of gut passage rates. This technique does not

allow us to assess the relative contribution of prey to an individual's diet, when a given taxa was ingested, or at what developmental stage (larval, adult) a given invertebrate was eaten (Bradley et al., 2007; King et al., 2008; Pompanon et al., 2012; Mallott et al., 2015). The lack of knowledge of gut passage rates of invertebrates in primates was especially apparent when we compared the taxa we observed individuals eating with the taxa present in their feces. Five fecal samples were collected within 24 hours of a behavioral record of that same individual eating an identified invertebrate. The Orders that were observed to be consumed were only present in one of the corresponding samples. In the other four samples, the sequences identified to at least the level of the Order did not include the observed taxa of prey that was consumed. Additionally, DNA barcoding of invertebrate mtDNA in the feces of primates does have the potential to detect secondary predation (invertebrates present in the gut of the consumed invertebrate), unintentionally consumed invertebrates (invertebrates in fruit or on leaves consumed by primates), and environmental contamination (from soil, leaf litter, or eggs laid on the surface of fecal samples) (King et al., 2008; Hofreiter et al., 2010; Pompanon et al., 2012; Mallott et al., 2015). Researchers must be mindful of these limitations and biases when employing DNA barcoding techniques.

Overall, the integration of molecular data on the taxa of invertebrates present in white-faced capuchin diets with both behavioral and ecological data has allowed us to gain a more in depth understanding of both the influence of food availability and sex- and age-based differences on prey foraging strategies in white-faced capuchins. The use of these molecular techniques, combined with field-based observational data, open up the possibility of examining in detail the role of faunivory in primate foraging strategies. Future studies including data on substrate and microhabitat preferences, more detailed studies of prey foraging techniques, and collection of

nutritional data for different invertebrate taxa will be informative to address questions of whether age-based differences are indeed due to differences in prey detection, extraction, or capture techniques, as our results suggest, or due to differences in nutrient demands.

Tables and Figures

	Overall (686.8 hours)	All Adults (n=10, 546 hours)	Adult Males (n=5, 217.8 hours)	Adult Females (n=5, 328.2 hours)	All Juveniles (n=12, 140.8 hours)	Juvenile Males (n=9, 82.3 hours)	Juvenile Females (n=3, 25.5 hours)
Feeding	12.62%	11.73%	13.07%	10.85%	15.11%	15.09%	15.45%
Foraging	26.33%	25.39%	22.16%	27.53%	31.42%	29.69%	31.15%
Resting	17.76%	17.66%	25.02%	12.78%	15.20%	17.85%	10.73%
Social	13.84%	14.40%	9.68%	17.53%	8.88%	8.06%	13.35%
Traveling	29.45%	30.82%	30.07%	31.31%	29.39%	29.31%	29.32%

Table 3.1. Activity budget of white-faced capuchins at La Suerte Biological Field Station, Costa Rica, from January 2013 – January 2014. Data from all adults and all juveniles also include records from individuals of unknown sex.

	Overall (267.9 hours)	All Adults (n=10, 202.6 hours)	Adult Males (n=5, 76.7 hours)	Adult Females (n=5, 125.9 hours)	All Juveniles (n=12, 65.4 hours)	Juvenile Males (n=9, 39.9 hours)	Juvenile Females (n=3, 11.9 hours)
Fruit	47.81%	48.15%	51.46%	46.13%	44.31%	45.36%	43.54%
Flowers	1.24%	1.10%	1.48%	0.88%	1.58%	2.59%	0%
Leaves	0.52%	0.49%	0.61%	0.42%	0.71%	0.33%	1.97%
Invertebrates	49.82%	49.61%	45.85%	51.91%	52.99%	51.30%	53.65%
Vertebrates	0.01%	0.02%	0.04%	0%	0.10%	0.17%	0%
Other	0.60%	0.63%	0.56%	0.66%	0.31%	0.25%	0.84%

Table 3.2. Diet of white-faced capuchins at La Suerte Biological Field Station, Costa Rica, from January 2013 – January 2014. Data from all adults and all juveniles also include records from individuals of unknown sex.

	Min/hr Feeding	Min/hr Foraging	Min/hr Feeding on Fruit	Min/hr Foraging on Fruit	Min/hr Feeding on Invertebrates	Min/hr Foraging on Invertebrates
Adults (n=10)	6.97 (±1.34)	15.38 (±3.24)	4.91 (±0.83)	6.51 (±2.91)	1.68 (±0.60)	8.72 (±2.50)
Juveniles (n=12)	10.03 (±2.14)	18.97 (±5.14)	6.78 (±2.24)	6.48 (±3.16)	2.53 (±0.94)	12.30 (±4.83)

Table 3.3. Time spent feeding and foraging on specific food types by age.

	Adult (n=10)	Juvenile (n=12)
Araneae	1.23%	18.18%
Blattodea	2.47%	0%
Hemiptera	13.58%	18.18%
Hymenoptera	28.40%	36.36%
Isoptera	16.05%	0%
Lepidoptera	7.41%	9.09%
Orthoptera	16.05%	9.09%
Phasmatodea	14.81%	9.09%

Table 3.4. Frequency of specific Orders of invertebrates in the diet by age using observational data (n=92 individual invertebrate feeding or foraging records where Order was identified, out of n=4048 invertebrate feeding and foraging observations).

	Adult (n=152)	Juvenile (n=73)
Lepidoptera	**43.04% (±20.96%)**	**61.26% (±12.54%)**
Diptera	35.47% (±20.24%)	52.07% (±28.48%)
Coleoptera	37.09% (±16.28%)	25.98% (±30.53%)
Hymenoptera	**20.42% (±13.13%)**	**44.04% (±26.56%)**
Hemiptera	28.19% (±13.11%)	15.28% (±17.18%)
Orthoptera	18.03% (±10.02%)	18.23% (±17.81%)

Table 3.5. Frequency of specific Orders of invertebrates in the diet by age using molecular data (n=225 fecal samples). Bolded numbers are significant at p=0.05.

	Adult (n=152) Observed	Expected	Juvenile (n=73) Observed	Expected	Concealed or embedded?	Highly mobile?
Araneae						
Salticidae	4	4.05	2	1.92	No	Yes
Blattodea						
Ectobiidae	1	9.46	4	4.48	Yes	No
Coleoptera						
Chrysomelidae	6	4.05	0	1.92	No	No
Curculionidae	20	16.89	5	8	Some	No
Elateridae	9	9.46	5	4.48	No	No
Diptera						
Cecidomyiidae	12	11.48	5	5.44	No	No
Culicidae	***1***	***5.4***	***7***	***2.56***	No	No
Tachinidae	6	6.08	3	2.88	No	No
Tenthredinidae	**2**	**4.05**	**4**	**1.92**	Some larvae	No
Tephritidae	17	17.56	9	8.32	No	No
Hemiptera						
Cercopidae	**19**	**14.86**	**3**	**7.04**	Nymphs	Yes
Fulgoridae	4	4.73	3	2.24	No	No
Hymenoptera						
Braconidae	3	2.7	1	1.28	No	No
Formicidae	17	16.89	8	8	No	No
Ichneumonidae	5	4.05	1	1.92	No	No
Lepidoptera						
Crambidae	**2**	**4.05**	**4**	**1.92**	Larvae	No
Geometridae	3	4.05	3	1.92	No	No
Hesperiidae	11	11.48	6	5.44	No	No
Notodontidae	5	4.05	1	1.92	No	No
Oecophoridae	4	3.38	1	1.6	Larvae	No
Pyralidae	4	3.38	1	1.6	Yes	No
Sphingidae	3	3.38	2	1.6	No	No
Tortricidae	**6**	**4.05**	**0**	**1.92**	Some larvae	No
Mantodea						
Liturgusidae	2	3.38	3	1.6	No	No
Mantidae	3	3.38	2	1.6	No	No
Orthoptera						
Acrididae	20	16.21	4	7.68	No	Yes
Gryllidae	**7**	**4.73**	**0**	**0.0224**	No	Yes
Plecoptera						
Perlodidae	4	3.38	1	1.6	No	No

Table 3.6. Observed versus expected frequency of occurrence by age of major arthropod families present in the diet based on molecular data. Bolded numbers are significant at p=0.1, bolded and italicized numbers are significant at p=0.05.

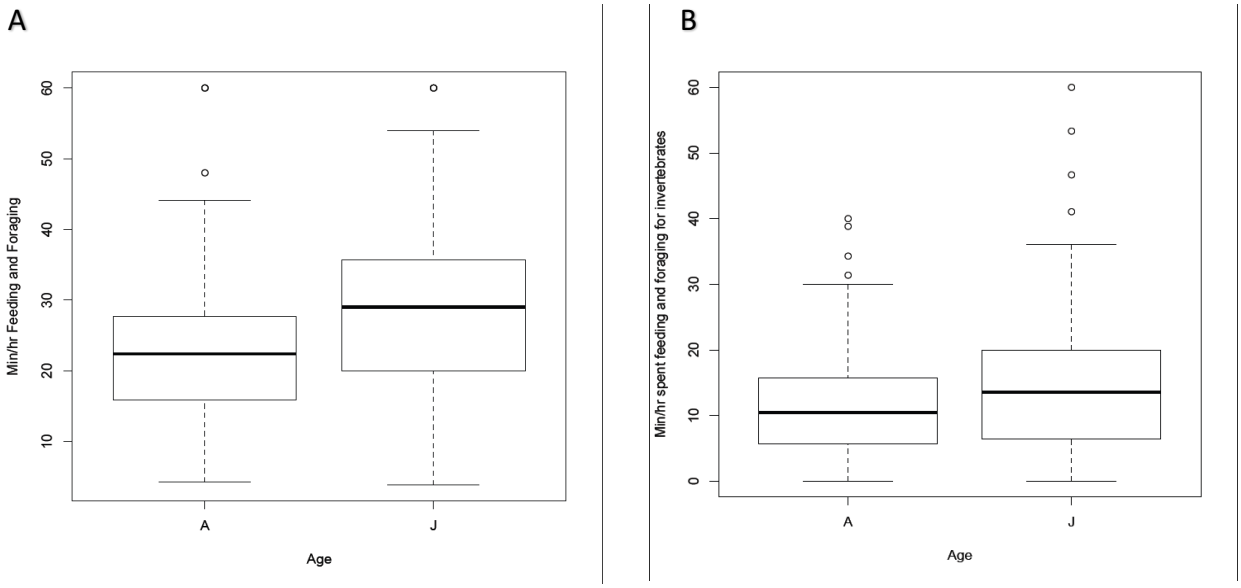

Figure 3.1. A: Adult (A) and juvenile (J) differences in minutes per hour spent feeding and foraging. Juveniles spent significantly more time feeding and foraging than adults. B: Adult (A) and juvenile (J) differences in minutes per hour spent feeding and foraging for invertebrates. Juveniles spent significantly more time foraging for invertebrates. Upper whisker = Q3+1.5*IQR and lower whisker = Q1-1.5*IQR or 0.

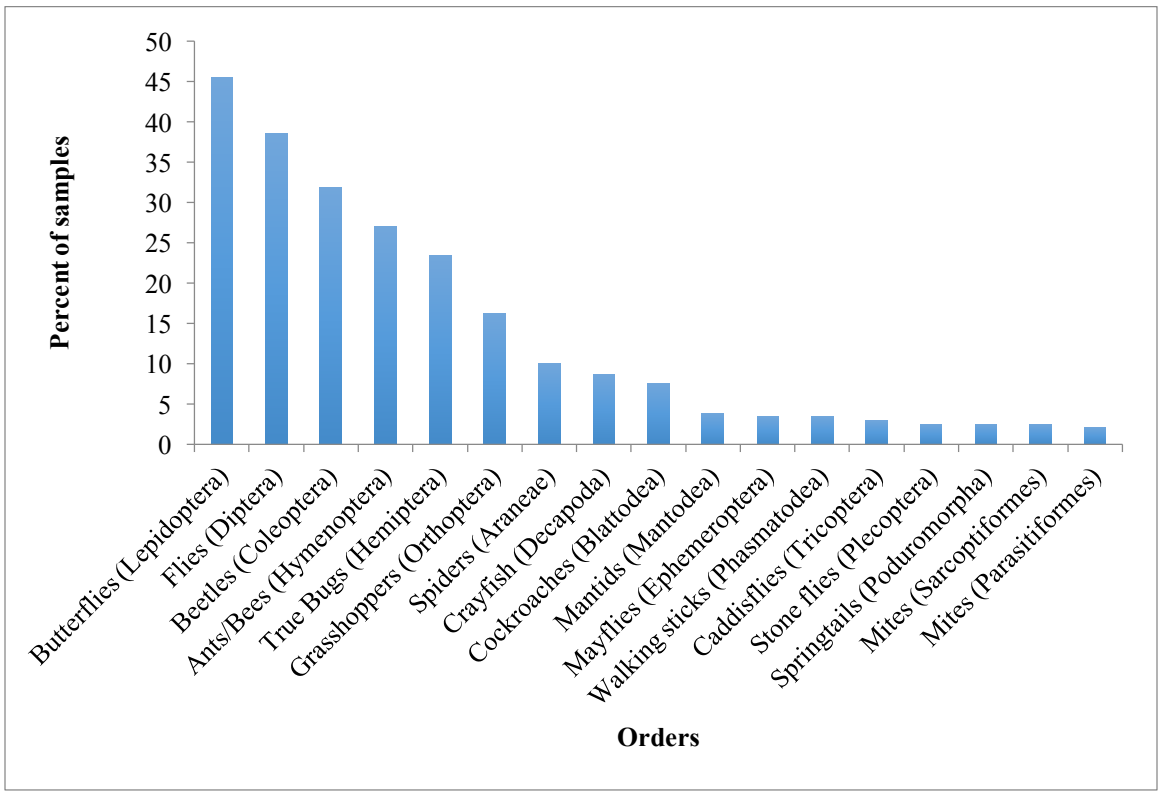

Figure 3.2. Frequency of arthropod Orders found in >2% of samples.

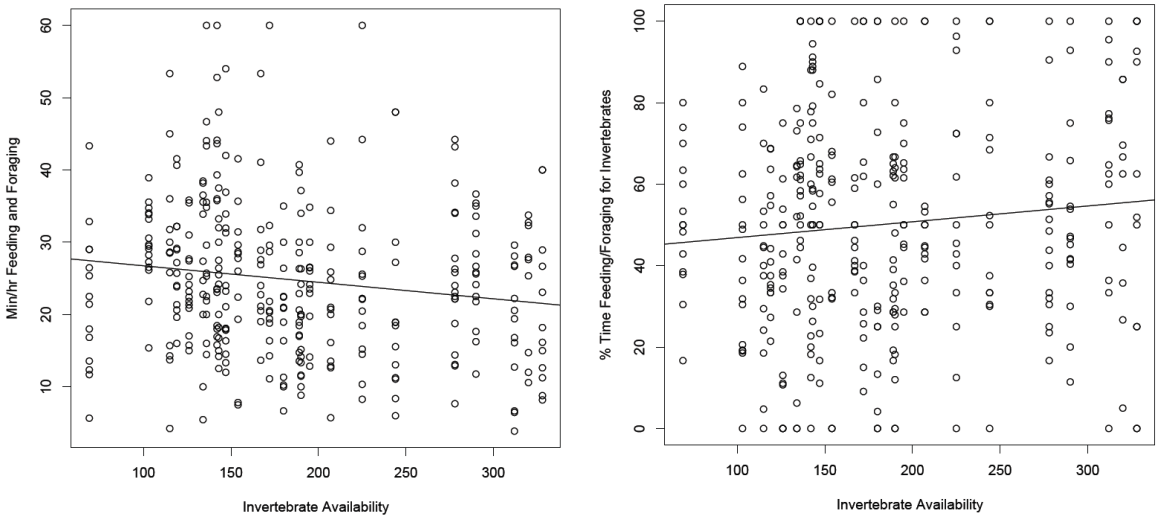

Figure 3.3. Influence of invertebrate availability on time spent feeding and foraging and influence of invertebrate availability on percentage of feeding and foraging time devoted to invertebrates. As invertebrate availability increased, white-faced capuchins spent significantly less time feeding and foraging and spent a significantly larger proportion of their feeding and foraging time on invertebrates.

CHAPTER 4

DIETARY CORRELATES OF GUT MICROBIOME COMPOSITION IN WILD WHITE-FACED CAPUCHINS (*CEBUS CAPUCINUS*)

Abstract

This study examines the response of white-faced capuchin gut microbial community structure and function to changes in diet in order to better understand how the gut microbiome buffers changes in nutrient availability and diet, and increases metabolic adaptability. During a 12-month period, information on foraging behavior was collected from wild white-faced capuchins. The V3-V5 region of microbial 16S rRNA was amplified from 169 fecal samples collected during the observational study. Samples were individually barcoded and sequenced on the Illumina MiSeq platform. OTUs were identified and assigned to taxa using the TORNADO pipeline. White-faced capuchin gut bacterial communities were characterized primarily by Firmicutes (41.6%) and Proteobacteria (39.2%). There was a significant effect of percentage of feeding and foraging time spent on fruit and invertebrates on community composition. Several plant and invertebrate taxa were significantly correlated with the number of OTUs assigned to predicted metabolic functions. The results of our study indicate that the minutes per hour spent consuming specific species of fruit and families of invertebrates may play a larger role in influencing gut microbiome function than the amount of time spent consuming broader categories, such as fruit.

Introduction

The gut microbiome plays an integral role in animal nutrition, digesting otherwise unavailable resources, providing substrates for nutrient metabolism in the gut, and increasing

nutrient uptake and utilization in the gut (Cummings and Macfarlane, 1997; Hooper et al., 2002). Many studies have shown that gut microbial community composition is related to differences in both macronutrient composition of the diet and the type of food consumed (e.g., fruit vs. leaves vs. animal prey), and that different gut microbial taxa can increase or decrease the amount of energy a host absorbs from a specific food (Hildebrandt et al., 2009; De Filippo et al., 2010; Wu et al., 2011; Claesson et al., 2012; Nelson et al., 2013). Despite a relatively robust understanding of how experimentally manipulated diet influences the gut microbiome in primates, with more plant-based diets being associated with increased relative abundance of Bacteriodetes and Acinetobacter and decreased relative abundance of Firmicutes and Proteobacteria (Ley et al., 2006b; Duncan et al., 2007; Kisidayová et al., 2009; Turnbaugh et al., 2009; Faith et al., 2011), the effect that changes in dietary choice on gut microbial community composition of wild populations of primates is not well studied (Amato, 2013b; Amato et al., 2013, 2014a; b; Gomez, 2014; Gomez et al., 2015).

Much of the intra- and interspecific variability in foraging behavior, dietary choice, ranging patterns, and grouping patterns seen in primates has been attributed to behavioral strategies that have evolved to address spatiotemporal variation in resource availability and nutritional challenges associated with feeding competition, predator avoidance, and age- and sex-based differences in nutrient needs (Wrangham, 1980; Chapman and Chapman, 1990; Van Schaik et al., 1993; O'Driscoll Worman and Chapman, 2005; Houle et al., 2007). Primate dietary choices are driven both by which foods are available and individual nutrient requirements. As a result, an individual's diet can undergo major shifts both in which types of food items are eaten (e.g. fruits, leaves, arthropods), and in which taxa of those food types are eaten over the course of days, weeks, and months (Chapman et al., 2003). Additionally, as both fruits and insects vary in

the amount of lipids, protein, sugars, complex polysaccharides, and chitin they contain, both between and within taxa, nutrient availability and consumption likely vary with spatiotemporal variation in food availability (Bell, 1990; Chivers, 1998; Chapman et al., 2003; O'Driscoll Worman and Chapman, 2005; Rothman et al., 2008; Raubenheimer and Rothman, 2013). Recent studies, both in primates and in other mammals, indicate that the gut microbiome buffer these dietary changes in response to changing food and nutrient availability (Tremaroli and Bäckhed, 2012; Amato, 2013a; Amato et al., 2014a; Schnorr et al., 2014; Gomez et al., 2015).

In this study, we examine how changes in fruit and invertebrate consumption over a 12-month period influence both gut microbial community structure and function in white-faced capuchins. White-faced capuchins (*Cebus capucinus*) are an excellent model for studying how variation of both fruit and invertebrate consumption influences the gut microbiome and how the gut microbiome may buffer dietary changes. White-faced capuchins are omnivorous, exploiting a wide range of food types. Based on several long term studies, 44.4% of feeding and foraging time was devoted to fruit, 3.4% to flowers, 2.7% to leaves, 9.5% to nuts and seeds, 38.0% to invertebrates, and 1.2% to vertebrates (Buckley, 1983; Rose, 1994; Baker, 1998; Urbani, 2009; McKinney, 2010). Variations in invertebrate prey choice may play a role that is equally important to variations in fruit foraging strategies in shaping white-faced capuchin nutritional strategies (Melin et al., 2014; Mosdossy et al., 2015), as capuchins exploit a more insectivorous diet than expected for their relatively large body size (2.61-3.97kg; Ford and Davis, 1992) (Kay and Simons, 1980), and are unusual among non-ape primates in that they are characterized by a large brain volume to body size ratio (72.95cc/3.29kg, n=23; Isler *et al.* 2008). Their reliance on animal prey appears to provide a readily digestible source of proteins and lipids necessary for growing and maintaining neural tissue (Aiello and Wheeler, 1995; Gunst et al., 2010;

Raubenheimer and Rothman, 2013). The gut microbiome may play an important role in compensating for variation in the percentage of feeding and foraging time spent on fruit and invertebrates, as rapid changes in gut microbial community structure can increase the uptake of specific nutrients, buffering changes in nutrient intake resulting from variation in resource availability (Hooper et al., 2002; Amato, 2013a; Amato et al., 2014a).

We combine behavioral data on foraging behavior and diet and DNA barcoding data on invertebrates in the diet with microbial analyses of fecal samples collected during a 12-month study of wild white-faced capuchins to address three predictions. As captive and wild studies of primates indicate that Bacteroidetes and Proteobacteria are positively correlated with the proportion of plants foods in the diet and Firmicutes and Acinetobacter are positively correlated with the proportion of animal foods in the diet (Turnbaugh et al., 2009; De Filippo et al., 2010; Amato et al., 2014a; Gomez et al., 2015), we expect that: **(1)** As the percentage of feeding and foraging time spent on fruits increases, the prevalence of Firmicutes and Proteobacteria taxa will decrease, while the prevalence of Bacteroidetes taxa will increase. **(2)** As the percentage of feeding and foraging time spent on arthropods increases, the prevalence of Firmicutes and Proteobacteria taxa will increase and the prevalence of Bacteroidetes taxa will decrease. **(3)** As changes in gut microbial community composition in response to diet are related to changes in gut microbial function, fruit consumption will be positively correlated with the number operational taxonomic units (OTUs) related to glycan and carbohydrate metabolism, which function in breaking down complex plant polysaccarides, while invertebrate consumption will be positively correlated with the number of OTUs related to amino acid and lipid metabolism due to their role in digesting animal prey.

Materials and Methods

Study site and population

Research was conducted from January 2013 through January 2014 at La Suerte Biological Field Station (LSBFS) in northeastern Costa Rica (10.445N, 83.784W). LSBFS contains approximately 300 ha of aseasonal wet, lowland tropical forest, including 170 ha of advanced secondary forest and 130 ha of early secondary growth and regenerating pasture (Garber and Paciulli, 1997). Four groups of *C. capucinus* are present, one of which is habituated. The group of habituated, individually recognizable *C. capucinus* that were used for the study contained 21-22 individuals (4 adult males, 5 adult females, 8-9 juvenile males, 2-3 juvenile females, and 0-2 infants) during the study period. All data collection methods were approved by the University of Illinois IACUC, La Suerte Biological Field Station, MINAET, SINAC, and CONAGEBIO.

Observational data

One-hour instantaneous focal animal samples (2-min interval) of individually recognizable adult males, adult females, juvenile males, and juvenile females were conducted 5 days a week (20 days/month) for 12 months. In total, 841 hours of behavioral data during 1341 hours of observation on 237 days were collected. Information on activity budget (feeding, foraging, traveling, resting, social, other), diet (ripe fruit, unripe fruit, invertebrates, vertebrates, flowers, leaves, seeds, other), and social interactions (including affiliative, aggressive, and submissive behaviors) was collected. When possible, consumed food items were identified to species (for fruit) or Order (for invertebrates). Not all juveniles were individually recognizable

during the early months of the study, so some juvenile focal follows were of individuals of unknown identity and sex.

Fecal samples

During the observational study, 225 fecal samples were collected from individually recognizable adult females (n=64), adult males (n=88), juvenile females (n=15), and juvenile males (n=47), as well as samples from juveniles of unknown sex (n=11). Fecal sample collection occurred throughout the day, but collection was biased towards the morning (145 morning samples vs. 80 afternoon samples; morning=0500-1100 and afternoon=1100-1700). Samples were collected in a manner to minimize contamination from exogenous sources, with care taken to avoid collecting material in contact with the forest floor or leaf litter. Samples were stored in 90% ethanol at -20C prior to being shipped to the Molecular Anthropology Laboratory at the University of Illinois at Urbana-Champaign.

DNA extraction

DNA was extracted from the fecal samples using a QIAamp DNA Stool Mini Kit following the provided protocol for "Isolation of DNA from Stool for Human DNA Analysis." Samples were homogenized using a vortexer prior to extraction. Modifications used by Pickett *et al.* (2012) were followed (see Mallott *et al.*, 2015 for detailed methods).

PCR amplification of arthropods

DNA barcoding of arthropod DNA in the fecal samples was used in order to determine the invertebrates consumed by white-faced capuchins. Two overlapping ~300bp fragments

within the COI mitochondrial gene were amplified from 56 of the 225 samples with universal arthropod primers using published protocols (Mallott et al., 2015). The second round of PCR amplification used fusion primers consisted of the original insect primers, plus individual sample barcodes and an adaptor sequence necessary for Illumina sequencing added to the 5' end of both the forward and reverse primers. Individually tagged amplicons were AMPure-bead purified, DNA concentration was quantified using a Qubit assay, and samples were diluted to provide an equal concentration in the pool to be sequenced.

The remaining 169 samples were amplified by polymerase chain reaction using PCR on a Fluidigm Access Array at the Roy J. Carver Biotechnology Center at UIUC. A mastermix for amplification was prepared using the Roche High Fidelity Fast Start Kit and 20x Access Array loading reagent according to Fluidigm protocols. To each well of a 48-well PCR plate, 3 µl mastermix, 1 µl DNA sample (2 ng/µl concentration), and 1 µl Fluidigm Illumina linkers with unique barcodes were added. In a separate plate, primer pairs were prepared and aliquoted. 20X primer solutions were prepared by adding 2 µl of each forward and reverse primer, 5 µl of Access Array Loading Reagent, and molecular grade H_2O to a final volume of 100 µl. 4 µl of sample was loaded in the sample inlets and 4 µl of primer solution was loaded in the primer inlets of a previously primed Fluidigm 48.48 Access Array IFC. The IFC was placed in an AX controller (Fluidigm Corp.) for microfluidic loading of all primer/sample combinations. Following the loading stage, the IFC plate was loaded on the Fluidigm Biomark HD PCR machine and samples were amplified using the following Access Array cycling program without imaging: 2 min at 50C, followed by 20 min at 70C, followed by 10 min at 95C, followed by 10 cycles of 15 sec at 95 C, 30 sec at 60C, 1 min at 72 C, followed by 2 cycles of 15 sec at 95C, 30 sec at 80C, 30 sec at 60C, 1 min at 72C, followed by 8 cycles of 15 sec at 95C, 30 sec at 60 C, 1

min at 72C, followed by 2 cycles of 15 sec at 95C, 30 sec at 80C, 30 sec at 60C, 1 min at 72C, followed by 8 cycles of 15 sec at 95C, 30 sec at 60 C, 1 min at 72C, followed by 5 cycles of 15 sec at 95C, 30 sec at 80 C, 30 sec at 60C, 1 min at 72 C. Following amplification, 2 µl of Fluidigm Harvest Buffer was loaded in the sample inlets and loaded on the AX controller for harvesting PCR products. Harvested product was then transferred to a new 96 well plate, quantitated on a Qubit, and all samples were run on a Fragment Analyzer (Advanced Analytics, Ames, IA) and amplicon regions and expected sizes were confirmed. Samples were then pooled based on product concentration, and were gel purified from a 2% agarose E-gel using a Qiagen gel extraction kit. All samples were individually barcoded and sequenced on the Illumina MiSeq platform.

Arthropod sequence processing and analysis

Raw sequence reads were demultiplexed and trimmed in QIIME (Caporaso et al., 2010). Reads were de-replicated, clustered at a 1% sequence divergence threshold, and chimeric sequences were detected denovo and removed in USEARCH (Edgar, 2010; Edgar et al., 2011). Sequence reads were clustered into OTUs using a 3% sequence divergence criteria in USEARCH. OTUs were compared to the GenBank nt nucleotide sequence database and the Barcode of Life Database (www.boldsystems.org) using BLAST (ncbi.nlm.nih.gov). OTUs were assigned to taxa in MEGAN5 using the standard settings (Huson et al., 2011).

PCR amplification of microbes

The V3-V5 region of the microbial 16S rRNA gene (forward primer: 5'-CCTACGGGAGGCAGCAG-3'; reverse primer: 5'-CCGTCAATTCMTTTRAGT-3') was

amplified from 169 samples by PCR on a Fluidigm Access Array at the Roy J. Carver Biotechnology Center at UIUC (see above for specific methods). Individually barcoded samples were then sequenced on the Illumina MiSeq platform at the Roy J. Carver Biotechnology Center at UIUC.

Microbial sequence processing and analysis

The IM-TORNADO pipeline (Sipos *et al.* 2010, version 2.0.3.1) was used to filter sequences, detect chimeras, trim and dereplicate sequences, and cluster sequences into OTUs at a 3% sequence divergence threshold. OTUs were assigned to taxonomy using the RDP database in IM-TORNADO. Functional predictions for each sample were made using PICRUST (Langille et al., 2013). The average weighted NSTI score across all 169 samples was 0.066±0.053 (range: 0.006-0.471).

Statistics

Permutational multivariate analysis of variance (PERMANOVA) was used to examine the influence of the percentage of feeding and foraging time devoted to fruit, and the percentage of feeding and foraging time devoted to invertebrates on gut microbial community composition, based on a Bray-Curtis dissimilarity matrix, including individual as a random effect to control for pseudoreplication (Adonis function, *vegan* package, r-project.org). Spearman correlations were used to assess the relationship of percentage of feeding and foraging time devoted to fruit, percentage of feeding and foraging time devoted to invertebrates, minutes per hour spent consuming individual fruit species (10 most frequently consumed species), and frequency of occurrence of invertebrate families in fecal samples (10 most frequently detected families) on the

relative abundance of microbial genera and the relative abundance of OTUs assigned to a particular KEGG pathway.

Results

Diet

Over the course of the 12-month study, 47.81% of feeding and foraging time was spent on fruit, 49.82% on invertebrates, 1.24% on flowers, 0.52% on leaves, 0.01% on vertebrates, and 0.60% on other plant foods. The group was observed eating 59 species of fruit during the study period, with 19 species being consumed in >0.5% of their fruit feeding and foraging records (Table 4.1). *Psidium guajava* (8.6%), *Hampea appendiculata* (3.9%), *Dipteryx panamensis* (3.4%), and *Inga spectabilis* (2.7%) dominated their diet. With the exception of *P. guajava*, all of these fruit were highly seasonal in their availability (Table 4.2). Twenty-nine Orders and 90 Families of arthropod were present in white-faced capuchin feces during the study period, with 23 Orders and 50 Families being identified in >1% of fecal samples and 9 Orders and 9 Families being identified in >5% of fecal samples (Table 4.2). Families of invertebrates that were frequently consumed by white-faced capuchins included Tephritidae (11.6%), Formicidae (11.1%), Curculionidae (11.1%), Acrididae (10.7%), and Cercopidae (9.8%) (Table 4.2). These values are based on the frequency of occurrence of each Order in the fecal samples from the molecular analysis and do not represent the proportion of insects consumed.

Gut microbial community structure

The white-faced capuchin gut microbiome was characterized by Firmicutes (41.6%), Proteobacteria (39.2%), and Bacteroidetes (13.3%) (Figure 4.1). The relative abundance of major

genera across all samples included *Clostridium XIVa* (16.4%), *Streptococcus* (13.2%), *Xylanibacter* (10.4%), *Actinobacillus* (7.5%), *Sutterella* (6.1%), *Megamonas* (5.6%), *Streptophyta* (4.4%), *Escherichia shigella* (4.1%), *Xanthomonas* (2.9%), and *Pseudomonas* (2.4%) (Appendix D).

There was no significant effect of percentage of feeding and foraging time spent on fruit or invertebrates on gut microbe community composition ($F_{1,142}$=0.533, R^2=0.003, p=0.950 and $F_{1,142}$=0.814, R^2=0.005, p=0.666) (Figure 4.2). The percentage of feeding and foraging time devoted to fruit was positively correlated with the relative abundance of OTUs assigned to *Clostridium XIVa* (Firmicutes) (ρ=0.127, p=0.100) and the percentage of feeding and foraging time devoted to invertebrates was negatively correlated with the relative abundance of *Clostridium XIVa* (Firmicutes) (ρ=-0.139, p=0.071); however, neither of these relationships were statistically significant. Overall, these data indicate that fruit foraging behavior has an opposite relationship with gut microbial taxa compared with invertebrate foraging behavior.

Minutes per hour spent consuming *Psidium guajava* were significantly negatively correlated with the relative abundance of OTUs assigned to *Xylanibacter* (Bacteroidetes) (ρ=-0.202, p=0.008) and *Clostridium XIVa* (Firmicutes) (ρ=-0.261, p<0.001) (Table 3). There was also a non-significant negative correlation between *Psidium guajava* and both *Sutterella* (Proteobacteria) (ρ=-0.139, p=0.070) and *Xanthomonas* (Proteobacteria) (ρ=-0.149, p=0.052) (Table 3). Time spent consuming *Hampea appendiculata* was significantly positively correlated with the relative abundance of OTUs assigned to *Xylanibacter* (Bacteroidetes) (ρ=0.206, p=0.007) and significantly negatively correlated with the relative abundance of *Clostridium XIVa* (Firmicutes) (ρ=-0.206, p=0.007) (Table 4.3). Minutes per hour spent consuming *Dipteryx panamensis* was significantly negatively correlated with the relative abundance of *Clostridium*

XIVa (Firmicutes) (ρ=-0.287, p<0.001), and had a non-significant negative relationship with *Sutterella* (Proteobacteria) (ρ=-0.133, p=0.083) (Table 4.3). Time spent consuming *Inga thibaudiana* was significantly positively correlated with the relative abundance of OTUs assigned to *Megamonas* (Firmicutes) (ρ=0.155, p=0.043) (Table 4.3). Time spent consuming *Inga spectabilis* was negatively correlated with the relative abundance of OTUs assigned to *Xylanibacter* (Bacteroidetes) (ρ=-0.139, p=0.028), but this relationship was not significant (Table 3). Increased consumption of fruit, such as *P. guajava* and *D. panamensis*, was significantly negatively correlated with the relative abundance of some genera in the phyla Firmicutes and Proteobacteria. Conversely, increases in the frequency of consumption of other fruit species (*H. appendiculata* and *I. thibaudiana*) were either positively correlated with the relative abundance of genera in the phylum Firmicutes, or were negatively correlated with some genera and not others. Overall, these results suggest that frequently consumed fruits are significantly influencing gut microbial community structure. The inverse relationship between the effects of different fruits we see here may be attributed to the fact that the statistical methods employed do not account for the fact that some of these fruit taxa are consumed in the same month.

The frequency of occurrence of Tephritidae (fruit flies) in white-faced capuchin feces was significantly positively correlated with the relative abundance of OTUs assigned to *Streptophyta* (Cyanobacteria) (ρ=0.182, p=0.018) (Table 4.4). The frequency of occurrence of Acrididae in fecal samples was significantly positively correlated with *Clostridium XIVa* (Firmicutes) (ρ=0.171, p=0.026), and there was a non-significant positive relationship between Acrididae and *Sutterella* (Proteobacteria) (ρ=0.144, p=0.060) (Table 4.4). Thus, as predicted, the

relatively abundance of Firmicutes increased as the frequency of consumption of some families of arthropods increased.

Gut microbial community function

The percentage of feeding and foraging time spent on fruit and the percentage of feeding and foraging time spent on invertebrates were not significantly correlated with the relative abundance of any functional pathways (all $p>0.05$). Minutes per hour spent consuming *P. guajava* was positively correlated with the relative abundance of OTUs assigned to glycan biosynthesis and metabolism pathways ($\rho=0.168$, $p=0.029$), and negatively correlated with the number of OTUs assigned to energy metabolism pathways ($\rho=-0.172$, $p=0.025$) and xenobiotics degradation and metabolism pathways ($\rho=-0.222$, $p=0.004$), partially supporting the predictions of our hypotheses. Time spent consuming *D. panamensis* was positively correlated with the relative abundance of OTUs assigned to amino acid metabolism pathways ($\rho=0.156$, $p=0.042$) and biosynthesis of other secondary metabolites pathways ($\rho=0.188$, $p=0.014$). Minutes per hour spent consuming *I. spectabilis* was positively correlated with the relative abundance of OTUs assigned to biosynthesis of other secondary metabolites pathways ($\rho=0.206$, $p=0.007$). The relationship between *D. panamensis* and *I. spectabilis* may indicate that these two Fabaceae species are high in tannins or other secondary metabolites. The frequency of occurrence of Formicidae in fecal samples was negatively correlated with the relative abundance of OTUs assigned to lipid metabolism pathways ($\rho=-0.167$, $p=0.030$). This was contrary to predictions that consumption of invertebrates would be positively correlated with the relative abundance of lipid metabolism pathways.

Discussion

This study examined the relationship between diet and the gut microbial community composition and function of white-faced capuchins. Changes in fruit consumption did not correspond to changes in gut microbial community structure. We also found no effect of invertebrate foraging behavior on overall gut microbial community structure. This may be due to the fact that invertebrate prey eaten by capuchins do not generally contain indigestible compounds, other than chitin, which capuchins possess the ability to digest (Stevens and Hume, 1995; Raubenheimer and Rothman, 2013).

However, there was an influence of both fruit and invertebrate foraging behavior on the relative abundance of individual microbial genera. The percentage of feeding and foraging time devoted to invertebrates was negatively correlated with taxa related to Firmicutes. This is contrast with the available data indicating that Firmicutes and Proteobacteria are more abundant when individuals consume lipid and animal protein rich foods sources (Turnbaugh and Gordon, 2009; De Filippo et al., 2010; Williams et al., 2013; Amato et al., 2014a; Gomez et al., 2015). The percentage of feeding and foraging time devoted to fruit was positively correlated with taxa related to Firmicutes, contrary to expectations based on the assumption that Firmicutes is less abundant in individuals consuming a higher percentage of plant foods. However, our results do support similar findings in wild western lowland gorillas, where groups with more frugivorous diets over a 2-month period had lower relative abundances of Bacteroidetes (17.9±5.2% vs. 28.2±8.2%) and higher relative abundances of Firmicutes (40.8±8.09% vs. 39±6.7%) (Gomez et al., 2015). Thus, the relationship between foraging behavior and the gut microbiome is perhaps dependent not only on the percentage of feeding and foraging time spent on broad categories of

foods (e.g. fruit or invertebrate), but on the specific taxa, actual amount of foods consumed, and nutritional content of food items.

Previous studies suggest that the relative abundance of genera related to Firmicutes and Proteobacteria are negatively correlated with the time spent consuming individual fruit species (Amato et al., 2014a). Our results support this, with consumption of several fruit species being negatively correlated with both genera related to Firmicutes and Proteobacteria, while arthropod families were positively correlated with the same genera. The exception to this is the positive correlation between *I. thibaudiana* and *Megamonas* (Firmicutes). The relationships between taxa related to Bacteroidetes and fruit consumption is not as clear, as there are negative correlations with two fruit species (*P. guajava* and *I. spectabilis*), but a positive correlation with a third (*H. appendiculata*). Though we predicted that all species of a particular food type would similarly influence gut microbial community structure and function, it is not unexpected that specific taxa of foods differ in their influence on the gut microbiome. Though we did not measure differences in the nutrient availability of foods, many authors have reported that not all invertebrates or fruits eaten by primate have the same nutrient content (Chivers, 1998; Chapman et al., 2003; O'Driscoll Worman and Chapman, 2005; Rothman et al., 2008; Raubenheimer and Rothman, 2013). Again, we emphasize that it is likely that broad dietary categories are not as important as the specific taxa of foods and types of plant tissues consumed in influencing the gut microbiome.

We confirmed that *P. guajava* consumption is positively correlated with glycan metabolizing pathways, as *P. guajava* has a relatively high neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose content compared with other fruit commonly eaten by white-faced capuchins (Eadie, 2012). In a study that measured the nutritional content of 22 fruits eaten by white-faced capuchins on the eastern coast of Costa Rica, mean NDF content for all

species of fruits was 26.0 % dry matter (range=7.3-54.1), ADF was 16.1 % dry matter (range=6.3-42.7), and cellulose was 9.8% dry matter (range 3.8-29.5) (Eadie, 2012). *P. guajava* had a NDF content of 54.1 % dry matter, ADF content of 42.7 % dry matter, and cellulose content of 29.5 % dry matter during this study (Eadie, 2012). Glycan metabolizing bacteria would allow white-faced capuchins to increase their digestive efficiency when relying heavily on *P. guajava*. Additionally, *P. guajava* has relatively less energy compared with other commonly consumed foods, with a caloric content of 178 kCal/100g, whereas average caloric content of all fruits was 365.6 kCal/100g (range=178-632 kCal/100g) (Eadie, 2012). If these values are similar to the values at our field site, we expect to see that energy metabolizing pathways would be negatively correlated with the consumption of this particular fruit. *P. guajava* is a staple food resource for this group and was one of the top three most commonly consumed fruit species in ten months of the year. We hypothesize that it plays a role in shaping gut microbial community composition and function in this population of white-faced capuchins. However, the functional data in this study should be interpreted cautiously, as we do not have data on digestive metabolites to corroborate the predicted functional pathways. Additionally, we did not measure actual nutrient consumption and there is no published data for all foods being consumed by individuals in our population. Even though we found relationships between the nutrient content of some fruits and gut microbial function, this does not represent a complete picture of how variation in macronutrient consumption influences white-faced capuchin gut microbial community structure and function.

It appears from our data that Proteobacteria is a major component of white-faced capuchin gut microbial communities and plays a large role in the digestive function of their gut microbiome. White-faced capuchins gut microbial community composition appears to be

different than that of most primates studied to date, with a much larger relative abundance of Proteobacteria and lower abundance of Bacteroidetes (Frey et al., 2006; Ley et al., 2008a; b; McKenna et al., 2008; Rezzi et al., 2009; Ochman et al., 2010; Szekely et al., 2010; Yildirim et al., 2010; Nakamura et al., 2011; Moeller et al., 2012, 2013b, 2015; Degnan et al., 2012; Amato et al., 2013; O'Sullivan et al., 2013; Amato et al., 2014b; Amato and Righini, 2015; Gomez et al., 2015; Hale et al., 2015); however, there are similarities to the gut microbiome of other primates that rely heavily on invertebrates (Bo et al., 2010; Xu et al., 2013). Pygmy lorises, whose diet is ~30% invertebrates (Starr and Nekaris, 2013), had similarly high relative abundances of Proteobacteria (30.43%), and, at the level of genus, high relative abundances of *Pseudomonas* and *Bacteroides* (Xu et al., 2013). In white-faced capuchins, *Bacteroides* had the third highest relative abundances of genera related to Bacteroidetes, and *Pseudomonas* was the genera with the fifth highest relative abundance within Proteobacteria (Table S2). In other species of mammals that regularly consume invertebrates, Proteobacteria also seem to figure prominently in their gut microbial communities; the gut microbiomes of myrmecophagous mammals are enriched for some taxa of Proteobacteria, including *Klebsiella*, compared with closely related non-myrmecophagous species (Delsuc et al., 2014).

The results of our study emphasize the necessity to sample the gut microbiome of the same primate species across a range of diets and environments in addition to across a diversity of taxa, allowing us to gain a more complete understanding of how environmental factors, including diet, influence the gut microbiome, informing studies of the co-evolution of gut microbes and host species (Amato, 2013a). Additional avenues for future research include sampling populations from the same species in different environments to control for host phylogenetic effects, and the addition of data on the nutrient content of the foods being consumed and

metabolomics data to provide a richer understanding of the complex relationship between environment factors, host species behavior, and gut microbial community composition and function.

Tables and Figures

Species	Family	% of diet	Months eaten
Psidium guajava	Myrtaceae	8.6%	All
Hampea appendiculata	Malvaceae	3.9%	Jan, Feb, Nov, Dec
Dipteryx panamensis	Fabaceae	3.4%	Jan - Jun
Inga spectabilis	Fabaceae	2.7%	Feb - May, Oct
Urera baccifera	Urticaceae	2.6%	Oct - Dec
Inga thibaudiana	Fabaceae	2.6%	Jan, Mar - May, Jul - Dec
Ficus colubrinae	Moraceae	1.7%	Feb, Mar, May - Aug, Oct - Dec
Ficus trigonata	Moraceae	1.5%	Apr, May, Oct, Nov
Conostegia xalapensis	Melastomataceae	1.4%	Jan, Apr - Jun, Aug, Sep, Nov, Dec
Sapium grandulosum	Euphorbiaceae	1.1%	Jul, Aug, Oct, Nov
Ficus schippii	Moraceae	0.9%	May, Jun, Nov
Inga marginata	Fabaceae	0.9%	Jan, Feb, Jun, Aug, Dec
Ficus tonduzii	Moraceae	0.7%	May, Oct
Nephelium lappaceum	Sapindaceae	0.7%	Jun - Oct
Cestrum megalophylum	Solanaceae	0.7%	Dec
Dendropanax arboreus	Araliaceae	0.6%	Sep, Oct
Piper spp.	Piperaceae	0.5%	Feb, Apr, Nov, Dec
Miconia affinis	Melastomataceae	0.5%	Apr - Jun, Aug, Sep
Casearia arborea	Salicaceae	0.5%	Jun, Oct, Nov

Table 4.1. Fruit species comprising >0.5% of white-faced capuchin fruit feeding and foraging time (n=17254 feeding and foraging records).

Order	Family	Number of samples	Frequency of occurrence
Amphipoda		**4**	**1.78%**
Araneae		**23**	**10.22%**
	Araneidae	3	1.33%
	Heptathelidae	4	1.78%
	Pisauridae	3	1.33%
	Salticidae	6	2.67%
Blattodea		**16**	**7.11%**
	Blaberidae	3	1.33%
	Blattidae	4	1.78%
	Ectobiidae	14	6.22%
Coleoptera		**72**	**32.00%**
	Chrysomelidae	6	2.67%
	Curculionidae	25	11.11%
	Elateridae	14	6.22%
	Staphylinidae	3	1.33%
Decapoda		**20**	**8.89%**
	Laomediidae	3	1.33%
Diptera		**87**	**38.67%**
	Agromyzidae	3	1.33%
	Anthomyiidae	4	1.78%
	Cecidomyiidae	17	7.56%
	Culicidae	8	3.56%
	Sciaridae	4	1.78%
	Tephritidae	26	11.56%
	Therevidae	3	1.33%
	Tachnidae	9	4.00%
Entomobryomorpha		**3**	**1.33%**
Ephemeroptera		**8**	**3.56%**
Hemiptera		**53**	**23.56%**
	Cercopidae	22	9.78%
	Cicadellidae	4	1.78%
	Flatidae	3	1.33%
	Fulgoridae	7	3.11%
	Reduviidae	3	1.33%
Hymenoptera		**61**	**27.11%**
	Apidae	3	1.33%
	Braconidae	4	1.78%
	Formicidae	25	11.11%
	Ichneumonidae	6	2.67%
	Tenthredinidae	6	2.67%
	Vespidae	4	1.78%

Table 4.2. Invertebrate species occurring in >1% of white-faced capuchin fecal samples.

Order	Family	Number of samples	Frequency of occurrence
Lepidoptera		**103**	**45.78%**
	Arctiidae	3	1.33%
	Crambidae	6	2.67%
	Geometridae	6	2.67%
	Gracillariidae	3	1.33%
	Hesperiidae	17	7.56%
	Noctuidae	4	1.78%
	Notodontidae	6	2.67%
	Oecophoridae	5	2.22%
	Pyralidae	5	2.22%
	Sphingidae	5	2.22%
	Tortricidae	6	2.67%
Mantodea		**9**	**4.00%**
	Liturgusidae	5	2.22%
	Mantidae	5	2.22%
Orthoptera		**37**	**16.44%**
	Acrididae	24	10.67%
	Gryllidae	7	3.11%
Parasitiformes		**5**	**2.22%**
Pendunculata		**3**	**1.33%**
	Lepadidae	3	1.33%
Phasmatodea		**8**	**3.56%**
	Phasmatidae	4	1.78%
Plecoptera		**6**	**2.67%**
	Perlodidae	5	2.22%
Poduromorpha		**6**	**2.67%**
	Hypogastruridae	3	1.33%
Sarcoptiformes		**6**	**2.67%**
Scorpiones		**3**	**1.33%**
Sessilia		**3**	**1.33%**
Tricoptera		**7**	**3.11%**
Trombidiformes		**4**	**1.78%**

Table 4.2 (cont.).

	Clostridium XlVa 83.6.	Streptococcus 100..2	Xylanibacter 79.	Actinobacillus 46.3	Sutterella 98.9.	Megamonas 100..2	Streptophyta 100..1	Xanthomonas 93.3	Escherichia Shigell 98.7.	Clostridium XlVa 58.3.
Psidium guajava	-0.111	0.102	-0.202**	-0.117	-0.139	-0.058	0.057	-0.149*	0.001	-0.216***
Hampea appendiculata	0.001	-0.059	0.207**	0.106	-0.007	0.064	0.009	-0.103	-0.079	-0.206**
Dipteryx panamensis	-0.101	0.086	-0.029	-0.029	-0.133	-0.032	-0.039	-0.093	0.044	-0.287***
Inga thibaudiana	0.003	-0.050	-0.028	-0.088	0.033	0.155*	-0.051	-0.005	-0.131	0.062
Inga spectabilis	-0.055	0.013	-0.139	-0.106	-0.047	-0.074	-0.003	0.026	0.128	-0.050
*=p<0.05, **=p<0.01, ***=p<0.001										

Table 4.3. Spearman correlation coefficients between the 10 most abundant microbial genera and 5 most frequently consumed fruit species. Starred results are significant correlations.

	Clostridium XlVa 83.6.	Streptococcus 100..2	Xylanibacter 79.	Actinobacillus 46.3	Sutterella 98.9.	Megamonas 100..2	Streptophyta 100..1	Xanthomonas 93.3	Escherichia Shigell 98.7.	Clostridium XlVa 58.3.
Tephritidae	0.025	0.027	-0.078	-0.008	-0.008	0.005	0.181*	-0.036	0.046	-0.026
Cercopidae	-0.093	-0.027	0.014	-0.113	-0.019	-0.102	-0.048	-0.008	-0.128	-0.110
Formicidae	0.063	-0.065	0.089	-0.126	0.035	0.008	0.026	0.019	0.019	0.025
Acrididae	0.088	-0.040	0.104	0.034	0.144	0.069	0.051	-0.059	0.007	0.0171*
Curculionidae	0.039	-0.048	0.055	-0.062	0.059	0.030	-0.041	0.005	-0.109	-0.009
*=p<0.05, **=p<0.01, ***=p<0.001										

Table 4.4. Spearman correlation coefficients between the 10 most abundant microbial genera and 5 most frequently consumed invertebrate families. Starred results are significant correlations.

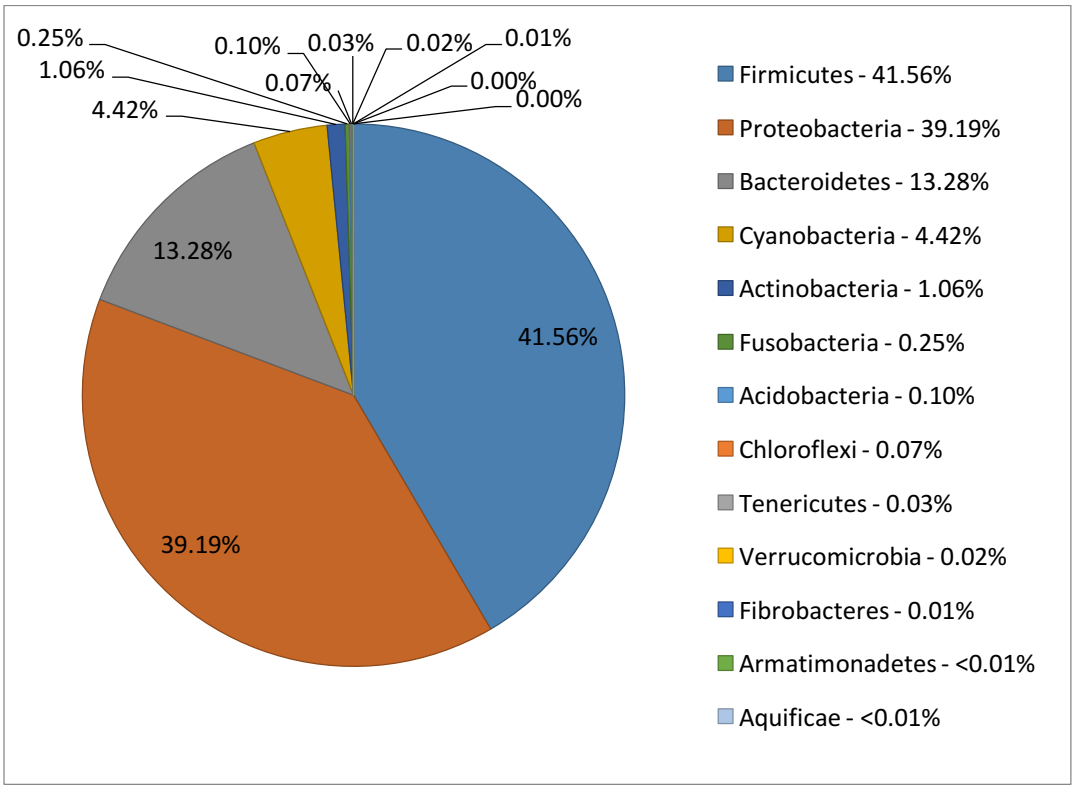

Figure 4.1. Relative abundance of Phyla found in the white-faced capuchin gut microbiome.

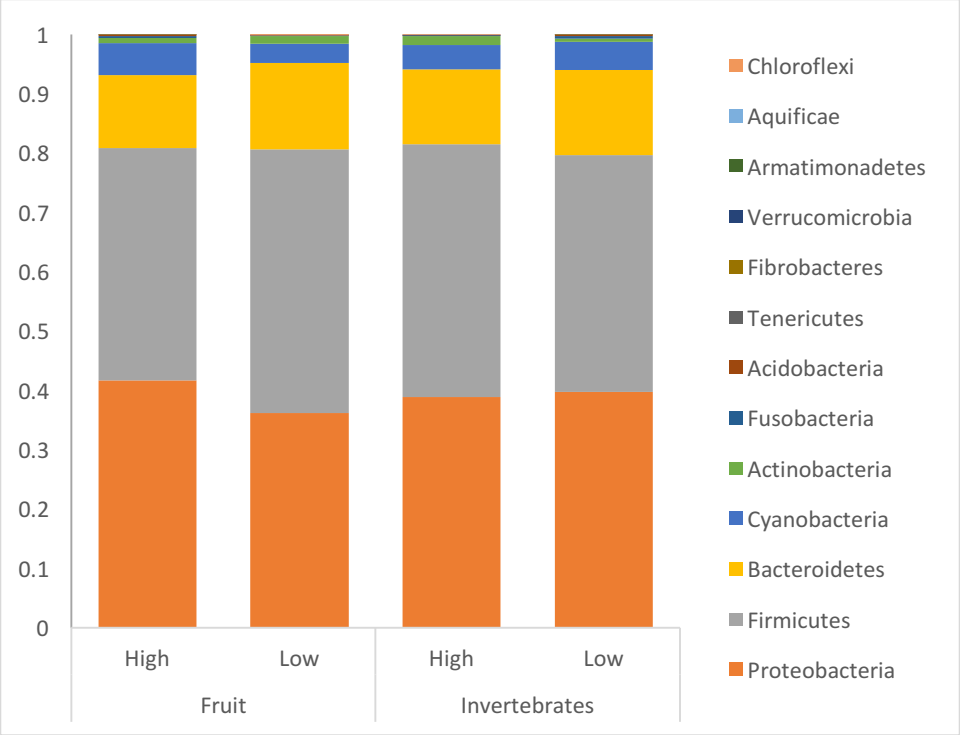

Figure 4.2. White-faced capuchin gut microbial community composition in periods of high (H) and low (L) percentage of feeding and foraging time spent on both fruit and invertebrates, averaged across all individuals.

CHAPTER 5

GENERAL CONCLUSIONS

This dissertation presents a multi-faceted approach to studying white-faced capuchin foraging ecology, and the findings underscore the importance of looking beyond food abundance and distribution as the primary factors driving nonhuman primate foraging strategies. We showed that the predictions of the socioecological model not are supported when considering the effects of fruit abundance and distribution, or invertebrate abundance and distribution on white-faced capuchin patterns of social spacing during foraging. This is not to say, however, that fruit and arthropod availability do not influence capuchin foraging strategies. It does suggest, however, that there are additional considerations when examining capuchin foraging strategies.

Fruit resources vary spatiotemporally in their nutritional content both between and within taxa of fruits being eaten, and several authors have suggested not treating fruit as a homogenous resource (Chivers, 1998; Chapman et al., 2003; O'Driscoll Worman and Chapman, 2005; Rothman et al., 2008). Along these same lines, lumping all arthropods into a broad category of invertebrate prey may not give us good understanding of how food resource availability impacts prey foraging strategies. Invertebrates are variable in nutrient content, particularly between different developmental stages of the same species (Bell, 1990; O'Malley and Power, 2012; Raubenheimer and Rothman, 2013). Additionally, different arthropod taxa are found in different microhabitats and can be solitary, colonial, social, or have variable distribution throughout their lifecycle (Bell, 1990). It is unlikely that the specific invertebrate prey capuchins are targeting can be viewed as a homogenously distributed resource. Thus, having a knowledge of the taxa of invertebrate primates are eating is essential for the wide range of primates for whom arthropods

are an essential part of their diets (Bartlett, 2011; Bogart and Pruetz, 2011; Di Fiore et al., 2011; Jaffe and Isbel, 2011; Thierry, 2011; Swedell, 2011; Shaffer, 2013), and this dissertation uses molecular methods to identify invertebrate prey present in nonhuman primate diets. Future studies should also consider how to collect data on the developmental stage (i.e., pupa, larva, adult) of invertebrates being eaten by primates.

Another important consideration when examining capuchin foraging strategies is that perhaps avoidance of feeding competition is not driving social relationships when primates are foraging for arthropods. Multiple authors have indicated that cooperative foraging models may be more informative (Janson and Boinski, 1992; Peres, 1992; Panger et al., 2002; Haugaasen and Peres, 2009). The results of this dissertation support this, as several measures of group cohesion increased when arthropod abundance decreased or invertebrate resources became more dispersed.

The second major finding of this dissertation is that juvenile and adult white-faced capuchins are employing different prey foraging strategies, emphasizing the role that development and ontogeny plays in individual responses to fluctuations in food availability. Based on the frequency of DNA from specific Orders of invertebrates in fecal samples, juvenile capuchins are eating less concealed, embedded, and highly mobile prey than adults are, but more larvae when compared with adults. These results indicating that juveniles have less developed prey foraging techniques than adults support the needing-to-learn hypothesis (Johnson and Bock, 2004), but, based on the results of this dissertation, it is unclear whether or not these age-based differences in dietary choice lead to differences in nutritional uptake. With their relatively long developmental period and expanded brain size (Fedigan and Rose, 1995; Jack and Fedigan,

2004; Isler et al., 2008; Perry et al., 2008; Muniz et al., 2010; Hartwig et al., 2011), capuchins will be an informative model to address this question in future studies.

Another significant finding of this dissertation is that white-faced capuchins eat a higher diversity of invertebrate prey when compared with many New World monkeys, but that their choice of invertebrate prey is not related to the abundance of those prey in the environment. The results presented in Chapter 4 show that proportion of fecal samples containing sequences for a specific Order of invertebrates in a two-week period has no significant relationship with the number of arthropods of that Order trapped in the same two-week period. Additionally, by comparing the rarified diversity of invertebrate prey found in this study to that found in seven other species of New World monkeys (Pickett et al., 2012; Mallott et al., 2015), we found that only squirrel monkeys (*Saimiri sciureus*) had a greater diversity of arthropod prey in their diet. Cebines exhibit a diverse set of invertebrate prey foraging strategies (Janson and Boinski, 1992; Panger et al., 2002; O'Malley and Fedigan, 2005; Gunst et al., 2010; Melin et al., 2014). It is likely that invertebrates are not only an important source of protein and lipids necessary to support the large brains of capuchins (Aiello and Wheeler, 1995; Gunst et al., 2010; Raubenheimer and Rothman, 2013), but that the expansion of cebid brain size allowed capuchins and squirrel monkeys to simultaneously more effectively exploit a wider range of prey and possibly target prey that are high in protein, lipid, and caloric content. Thus, studies of the diversity of prey foraging strategies across primates may give us additional insight into concepts such as cognitive buffering and niche construction (Mackinnon and Fuentes, 2011; Van Woerden et al., 2011; MacKinnon and Fuentes, 2012; van Woerden et al., 2014).

The fourth major finding of this dissertation is that the function of the gut microbiome of white-faced capuchins responds to changes in their diet and likely increases the uptake of

nutrients from less optimal food resources. For example, guava (*Psidium guava*) is comparatively high in cellulose content and low in caloric content. When white-faced capuchins are spending a greater percentage of their feeding and foraging time on guava, the relative abundance of functional pathways related to cellulose metabolism increase. While previous research has highlighted the role of the gut microbiome in nutrient uptake and buffering dietary changes in primates (Cummings and Macfarlane, 1997; Hooper et al., 2002; Hildebrandt et al., 2009; De Filippo et al., 2010; Wu et al., 2011; Claesson et al., 2012; Nelson et al., 2013), little was known about how the nutrient composition of individual taxa of foods influenced gut microbial community structure and function. Additionally, a substantial proportion of the variation in gut microbial community structure (16%) in white faced capuchins can be explained by variation between individuals. These results point to the importance of individual physiological, genetic, and behavioral differences in shaping primate foraging strategies.

The major findings of this dissertation indicate that the socioecological model may not be sufficient to explain primate sociality and foraging ecology. The socioecological model proposes that group living requires balancing the costs of feeding competition with the benefits of predator avoidance and access to reproductive partners (Wrangham, 1980; Van Schaik and Van Hooff, 1983; Terborgh and Janson, 1986; Sterck et al., 1997; Chapman and Chapman, 2000). This model posits that food availability determine social interactions, proximity relationships, kinship, and foraging behavior (in ways that are age-, sex-, or dominance-dependent), which then influence foraging success and differences in fitness (Wrangham, 1980; Terborgh and Janson, 1986; Sterck et al., 1997; Snaith and Chapman, 2007). However, this model makes two assumptions that are likely not strictly true – that there is an optimal strategy for dealing with all possible food availability situations, and that an individual will follow the optimal strategy. In

reality, there are likely a myriad of strategies to exploit foods, there may be many peaks in any given fitness landscape, and most primates do not live in food-limiting environments. Social foraging theory describes three primary foraging strategies: finders – individuals that search for patches and enter a patch first, and joiners – individuals that monitor the interactions of finders and join or supplant other individuals from already occupied patches (Giraldeau and Caraco, 2000). However, in reality, most individuals are opportunists, flexibly switching strategies based on current ecological, social, and intrinsic conditions (Giraldeau and Caraco, 2000; Morand-Ferron et al., 2011). Models of primate foraging strategies should include not only ecological and social information, but also individual-level factors such as physiology, personality, genetic traits, and commensal microbial relationships (Dammhahn and Almeling, 2012; Tanner and Jackson, 2012; Amato, 2013a; Dosmann and Mateo, 2014).

The various methodologies used in this dissertation were not without limitations. Our methods of measuring invertebrate abundance likely under-sampled embedded and more sedentary invertebrates, decreasing our ability to measure the abundance of invertebrate taxa actually present in the diet of white-faced capuchins. Additionally, the DNA barcoding method used to assess the invertebrates present in the diet is limited by primer biases, amplification stocasticity, and lack of knowledge of gut passage rates. Additionally, DNA barcoding only allows us to identify the taxa of a prey item being consumed, not at what developmental stage the taxa is or the quantity ingested of a given taxon. Future studies of foraging strategies in white-faced capuchins would be strengthened by the additional of data on the nutritional content of foods eaten and directly measuring gut microbial function, either by assessing the metabolites or RNA sequences present in the feces.

This dissertation provides a multi-level approach to studying primate foraging ecology, integrative behavioral, ecological, and molecular data to examine foraging strategies using ecological, social, and intrinsic factors. It also presents the first data on white-faced capuchin gut microbial communities, greatly expands our knowledge of capuchin invertebrate prey, and provides a comprehensive dataset on white-faced capuchin foraging ecology in a tropical wet forest. The integrative multifaceted approach to primate foraging ecology provides a framework with which to begin truly understanding the complexity and plasticity of primate foraging strategies.

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APPENDIX A

ACTIVITY BUDGET OF *CEBUS CAPUCINUS* AT LA SUERTE BIOLOGICAL FIELD STATION FROM FEBRUARY 2013 to JANUARY 2014

Month	Feeding	Foraging	Resting	Social	Traveling
February	11.45%	31.43%	12.24%	11.20%	33.68%
March	14.83%	30.93%	14.42%	14.62%	25.20%
April	11.76%	27.98%	17.90%	13.28%	29.08%
May	8.67%	26.52%	25.75%	17.13%	21.93%
June	9.89%	20.05%	20.87%	13.88%	35.31%
July	15.29%	20.17%	20.04%	13.12%	31.38%
August	13.28%	23.47%	17.24%	14.76%	31.25%
September	13.94%	20.83%	20.71%	16.87%	27.65%
October	14.74%	21.01%	18.86%	17.33%	28.06%
November	16.66%	27.74%	15.03%	7.31%	33.26%
December	10.70%	28.83%	15.53%	12.94%	32.00%
January	9.40%	38.57%	13.46%	14.31%	24.26%
Overall	12.62%	26.33%	17.76%	13.84%	29.45%

APPENDIX B

DIET OF *CEBUS CAPUCINUS* AT LA SUERTE BIOLOGICAL FIELD STATION FROM FEBRUARY 2013 TO JANUARY 2014

	Ripe fruit	Unripe fruit	Flowers	Seeds	Leaves	Invertebrates	Vertebrates	Other
February	41.95%	0.00%	0.85%	0.00%	0.28%	55.23%	0.00%	1.69%
March	50.96%	0.00%	0.00%	0.00%	0.44%	48.16%	0.00%	0.44%
April	47.16%	0.00%	0.53%	0.00%	1.06%	50.86%	0.00%	0.39%
May	53.46%	0.00%	0.00%	0.16%	0.47%	45.75%	0.00%	0.16%
June	30%	0.91%	1.52%	0.00%	0.00%	67.27%	0.00%	0.30%
July	43.31%	3.53%	2.97%	0.00%	0.56%	49.07%	0.00%	0.56%
August	47.26%	0.73%	3.28%	0.00%	0.00%	47.81%	0.00%	0.92%
September	40.21%	3.15%	0.70%	0.00%	0.00%	54.72%	0.17%	1.05%
October	59.72%	0.47%	1.26%	0.00%	0.47%	37.60%	0.00%	0.48%
November	52.87%	0.13%	2.22%	0.00%	0.65%	43.60%	0.00%	0.53%
December	47.31%	0.00%	1.05%	0.00%	1.45%	49.80%	0.00%	0.39%
January	41.52%	0.00%	0.88%	0.00%	0.00%	57.60%	0.00%	0.00%
Overall	47.16%	0.65%	1.24%	0.01%	0.52%	49.82%	0.01%	0.59%

APPENDIX C

INVERTEBRATE ORDERS, FAMILIES, GENERA, AND SPECIES PRESENT IN THE DIET OF *CEBUS CAPUCINUS* AT LA SUERTE BIOLOGICAL FIELD STATION FROM JANUARY 2013 to JANUARY 2014 DETERMINED USING MOLECULAR DATA

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
Amphipoda			**6**	**0.018%**	**4**	**1.78%**
	Gammaridae		1	0.003%	1	0.44%
		Gammarus pulex	*1*	*0.003%*	*1*	*0.44%*
	Lysianassidae		1	0.003%	1	0.44%
		Orchomenyx tabarini	*1*	*0.003%*	*1*	*0.44%*
Araneae			**186**	**0.565%**	**23**	**10.22%**
	Araneidae		10	0.030%	3	1.33%
		Araneus	*2*	*0.006%*	*1*	*0.44%*
		Micrathena	*4*	*0.012%*	*1*	*0.44%*
	Clubionidae		1	0.003%	1	0.44%
		Clubiona	*1*	*0.003%*	*1*	*0.44%*
	Gnaphosidae		3	0.009%	1	0.44%
		Gnaphosa	*3*	*0.009%*	*1*	*0.44%*
	Heptathelidae		19	0.058%	4	1.78%
		Ryuthela	*19*	*0.058%*	*4*	*1.78%*
		Ryuthela sasakii	*18*	*0.055%*	*4*	*1.78%*
		Ryuthela ishigakiensis	*1*	*0.003%*	*1*	*0.44%*
	Leptonetidae		2	0.006%	1	0.44%
		Neoleptoneta georgia	*2*	*0.006%*	*1*	*0.44%*
	Linyphiidae		1	0.003%	1	0.44%
		Pocadicnemis pumila	*1*	*0.003%*	*1*	*0.44%*
	Lycosidae		5	0.015%	1	0.44%
		Pirata insularis	*5*	*0.015%*	*1*	*0.44%*
	Phalangodidae		1	0.003%	1	0.44%
		Pseudobiantes japonicus	*1*	*0.003%*	*1*	*0.44%*
	Pisauridae		6	0.018%	3	1.33%
		Dolomedes	*6*	*0.018%*	*3*	*1.33%*
		Dolomedes pegasus	*4*	*0.012%*	*1*	*0.44%*
	Salticidae		35	0.106%	6	2.67%
		Orthrus bicolor	*30*	*0.091%*	*6*	*2.67%*
	Thomisidae		1	0.003%	1	0.44%
		Ozyptila gertschi	*1*	*0.003%*	*1*	*0.44%*
Archaeognatha			**2**	**0.006%**	**1**	**0.44%**
Blattodea			**993**	**3.015%**	**16**	**7.11%**
	Blaberidae		26	0.079%	3	1.33%
		Phoetalia pallida	*3*	*0.009%*	*2*	*0.89%*
		Rhabdoblatta	*22*	*0.067%*	*1*	*0.44%*
		Rhabdoblatta formosana	*21*	*0.064%*	*1*	*0.44%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Blattodea)	Blattidae		200	0.607%	4	1.78%
		Celatoblatta	*7*	*0.021%*	*1*	*0.44%*
		Melanozosteria	*184*	*0.559%*	*4*	*1.78%*
	Ectobiidae		373	1.133%	14	6.22%
		Blatella	*12*	*0.036%*	*1*	*0.44%*
		Blatella germanica	*6*	*0.018%*	*1*	*0.44%*
		Loboptera decipiens	*1*	*0.003%*	*1*	*0.44%*
		Neoblattella	*6*	*0.018%*	*2*	*0.89%*
Coleoptera			**2653**	**8.056%**	**72**	**32.00%**
	Anthicidae		7	0.021%	2	0.89%
		Anthicus	7	0.021%	2	0.89%
	Brachyceridae		3	0.009%	1	0.44%
		Catasarcus	*3*	*0.009%*	*1*	*0.44%*
	Carabidae		1	0.003%	1	0.44%
		Clivina	*1*	*0.003%*	*1*	*0.44%*
	Chrysomelidae		6	0.018%	6	2.67%
		Callosobruchus nigripennis	*1*	*0.003%*	*1*	*0.44%*
		Cassida nebulosa	*1*	*0.003%*	*1*	*0.44%*
		Cephaloleia suaveola	*1*	*0.003%*	*1*	*0.44%*
		Epitrix hirtipennis	*1*	*0.003%*	*1*	*0.44%*
		Pachymerus cardo	*1*	*0.003%*	*1*	*0.44%*
	Curculionidae		59	0.179%	25	11.11%
		Calacalles kabylianus	*1*	*0.003%*	*1*	*0.44%*
		Curculio arakawai	*2*	*0.006%*	*1*	*0.44%*
		Dendroterus defectus	*1*	*0.003%*	*1*	*0.44%*
		Diaprepes	*1*	*0.003%*	*1*	*0.44%*
		Exophthalmus roseipes	*18*	*0.055%*	*2*	*0.89%*
		Glostatus	*4*	*0.012%*	*2*	*0.89%*
		Porthetes	*9*	*0.027%*	*1*	*0.44%*
		Scolytus	*1*	*0.003%*	*1*	*0.44%*
		Sitophilus zeamais	*1*	*0.003%*	*1*	*0.44%*
		Sphenophorus levis	*1*	*0.003%*	*1*	*0.44%*
		Strophosoma melanogrammum	*1*	*0.003%*	*1*	*0.44%*
		Tanymecus	*1*	*0.003%*	*1*	*0.44%*
		Trigonopterus	*2*	*0.006%*	*2*	*0.89%*
		Xylosandrus germanus	*13*	*0.039%*	*11*	*4.89%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Coleoptera)	Elateridae		1668	5.065%	14	6.22%
		Campsosternus argentipilis	*1*	*0.003%*	*1*	*0.44%*
		Dalopius	*1641*	*4.983%*	*10*	*4.44%*
		Denticollis	*2*	*0.006%*	*1*	*0.44%*
		Melanotus	*8*	*0.024%*	*1*	*0.44%*
		Sericus	*1*	*0.003%*	*1*	*0.44%*
	Hydraenidae		1	0.003%	1	0.44%
		Hydraena	*1*	*0.003%*	*1*	*0.44%*
	Kateretidae		5	0.015%	1	0.44%
		Kateretes rufilabris	*5*	*0.015%*	*1*	*0.44%*
	Leiodidae		3	0.009%	1	0.44%
		Catops coracinus	*3*	*0.009%*	*1*	*0.44%*
	Staphylinidae		3	0.009%	3	1.33%
		Stenus palustris	*1*	*0.003%*	*1*	*0.44%*
	Tenebrionidae		1	0.003%	1	0.44%
		Branchus	*1*	*0.003%*	*1*	*0.44%*
Decapoda			**1068**	**3.243%**	**20**	**8.89%**
	Alpheidea		28	0.085%	1	0.44%
		Synalpheus belizensis	*28*	*0.085%*	*1*	*0.44%*
	Atyidae		1	0.003%	1	0.44%
		Halocaridina rubra	*1*	*0.003%*	*1*	*0.44%*
	Cambaridae		3	0.009%	2	0.89%
		Cambarus	*2*	*0.006%*	*1*	*0.44%*
		Orconectes mirus	*1*	*0.003%*	*1*	*0.44%*
	Cryptochiridae		8	0.024%	1	0.44%
	Diogenidae		1	0.003%	1	0.44%
		Dardanus fucosus	*1*	*0.003%*	*1*	*0.44%*
	Epialtidae		1	0.003%	1	0.44%
		Epialtus	*1*	*0.003%*	*1*	*0.44%*
	Galatheidae		958	2.909%	1	0.44%
		Munidopsis spinifer	*958*	*2.909%*	*1*	*0.44%*
	Laomediidae		16	0.049%	3	1.33%
		Laomedia astacina	*16*	*0.049%*	*3*	*1.33%*
	Linnaeoxanthidae		16	0.049%	1	0.44%
	Nephropidae		1	0.003%	1	0.44%
		Metanephrops sinensis	*1*	*0.003%*	*1*	*0.44%*
	Paguridae		2	0.006%	1	0.44%
		Pagurus pollicaris	*2*	*0.006%*	*1*	*0.44%*
	Palaemonidae		2	0.006%	1	0.44%
		Macrobrachium rosenbergii	*2*	*0.006%*	*1*	*0.44%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Decapoda)	Pandalidae		1	0.003%	1	0.44%
		Pandalus danae	*1*	*0.003%*	*1*	*0.44%*
	Parathelphusidae		1	0.003%	1	0.44%
		Parathelphusa	*1*	*0.003%*	*1*	*0.44%*
	Processidae		2	0.006%	1	0.44%
		Processa modica	*2*	*0.006%*	*1*	*0.44%*
Diptera			**4588**	**13.933%**	**87**	**38.67%**
	Acroceridae		1	0.003%	1	0.44%
	Agromyzidae		3	0.009%	3	1.33%
		Chromatomyia horticola	*1*	*0.003%*	*1*	*0.44%*
		Phytomyza notata	*1*	*0.003%*	*1*	*0.44%*
	Anthomyiidae		4	0.012%	4	1.78%
		Delia platura	*1*	*0.003%*	*1*	*0.44%*
		Lasiomma	*2*	*0.006%*	*2*	*0.89%*
		Lasiomma latipenne	*1*	*0.003%*	*1*	*0.44%*
		Pegomya	*1*	*0.003%*	*1*	*0.44%*
	Cecidomyiidae		110	0.334%	17	7.56%
		Asteromyia	*1*	*0.003%*	*1*	*0.44%*
		Contarinia	*9*	*0.027%*	*3*	*1.33%*
		Dasineura	*1*	*0.003%*	*1*	*0.44%*
		Sitodiplosis mosellana	*6*	*0.018%*	*2*	*0.89%*
	Ceratopogonidae		2	0.006%	2	0.89%
	Chloropidae		1	0.003%	1	0.44%
		Oscinella	*1*	*0.003%*	*1*	*0.44%*
	Culicidae		14	0.043%	8	3.56%
		Anopheles	*8*	*0.024%*	*2*	*0.89%*
		Culex pipiens	*5*	*0.015%*	*5*	*2.22%*
	Dolichopodidae		4	0.012%	2	0.89%
	Drosophilidae		1	0.003%	1	0.44%
		Drosophila tristis	*1*	*0.003%*	*1*	*0.44%*
	Heleomyzidae		3	0.009%	2	0.89%
	Muscidae		3	0.009%	2	0.89%
		Coenosia comita	*1*	*0.003%*	*1*	*0.44%*
	Mycetophilidae		1	0.003%	1	0.44%
		Mycetophila	*1*	*0.003%*	*1*	*0.44%*
	Pallopteridae		1	0.003%	1	0.44%
	Phoridae		2	0.006%	1	0.44%
	Psychodidae		2	0.006%	1	0.44%
		Lutzomyia gomezi	*1*	*0.003%*	*1*	*0.44%*
	Scathophagidae		2	0.006%	1	0.44%

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Diptera)	Sciaridae		5	0.015%	4	1.78%
	Simuliidae		1	0.003%	1	0.44%
		Simulium silvestre	*1*	*0.003%*	*1*	*0.44%*
	Sphaeroceridae		1	0.003%	1	0.44%
		Spelobia	*1*	*0.003%*	*1*	*0.44%*
	Stratiomyidae		1	0.003%	1	0.44%
		Stratiomys singularior	*1*	*0.003%*	*1*	*0.44%*
	Syrphidae		9	0.027%	2	0.89%
		Cheilosia	*7*	*0.021%*	*1*	*0.44%*
		Dasysyrphus albostriatus	*1*	*0.003%*	*1*	*0.44%*
	Tabanidae		1	0.003%	1	0.44%
		Chrysops	*1*	*0.003%*	*1*	*0.44%*
	Tephritidae		3397	10.316%	26	11.56%
		Anastrepha	*3397*	*10.316%*	*26*	*11.56%*
	Therevidae		3	0.009%	3	1.33%
	Tachnidae		353	1.072%	9	4.00%
		Belvosia	*7*	*0.021%*	*2*	*0.89%*
		Blepharipa fimbriata	*1*	*0.003%*	*1*	*0.44%*
		Drino	*327*	*0.993%*	*2*	*0.89%*
		Houghia	*4*	*0.012%*	*1*	*0.44%*
		Leschenaultia	*2*	*0.006%*	*1*	*0.44%*
		Pseudochaeta	*4*	*0.012%*	*2*	*0.89%*
Entomobryomorpha			**857**	**2.602%**	**3**	**1.33%**
	Entomybryidae		78	0.237%	1	0.44%
		Entomobrya marginata	*42*	*0.128%*	*1*	*0.44%*
		Entomobrya multifasciata	*2*	*0.006%*	*1*	*0.44%*
		Lepidocyrtus	*26*	*0.079%*	*1*	*0.44%*
		Lepidocyrtus cyaneus	*1*	*0.003%*	*1*	*0.44%*
	Isotomidae		33	0.100%	1	0.44%
		Folsomia quadrioculata	*29*	*0.088%*	*1*	*0.44%*
		Isotomurus plumosus	*1*	*0.003%*	*1*	*0.44%*
	Paronellidae		30	0.091%	1	0.44%
	Tomoceridae		44	0.134%	1	0.44%
		Pogonognathellus longicornus	*3*	*0.009%*	*1*	*0.44%*
		Tomocerus nigrus	*1*	*0.003%*	*1*	*0.44%*
Ephemeroptera			**13**	**0.039%**	**8**	**3.56%**
	Ameletidae		1	0.003%	1	0.44%
		Amerletus oregonenesis	*1*	*0.003%*	*1*	*0.44%*
	Baetidae		4	0.012%	2	0.89%
		Baetis pseudorhodani	*4*	*0.012%*	*2*	*0.89%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
Euphausiacea			**2**	**0.006%**	**1**	**0.44%**
	Euphausiidae		2	0.006%	1	0.44%
		Euphausia tenera	2	0.006%	1	0.44%
Hemiptera			**4590**	**13.939%**	**53**	**23.56%**
	Achilidae		2	0.006%	1	0.44%
	Aleyrodidae		2	0.006%	2	0.89%
		Bemisia tabaci	*2*	*0.006%*	*2*	*0.89%*
	Alydidae		1	0.003%	1	0.44%
		Nariscus	*1*	*0.003%*	*1*	*0.44%*
	Aphididae		6	0.018%	2	0.89%
		Aphis gossypii	*1*	*0.003%*	*1*	*0.44%*
		Pentalonia nigronervosa	*5*	*0.015%*	*1*	*0.44%*
	Aphrophoridae		6	0.018%	2	0.89%
		Aphrophora fulva	*3*	*0.009%*	*1*	*0.44%*
		Cephisus siccifolius	*3*	*0.009%*	*1*	*0.44%*
	Cercopidae		2025	6.149%	22	9.78%
		Mahanarva costaricensis	*2025*	*6.149%*	*22*	*9.78%*
	Cicadellidae		5	0.015%	4	1.78%
		Cetexa	*2*	*0.006%*	*1*	*0.44%*
		Flexamia flexulosa	*1*	*0.003%*	*1*	*0.44%*
		Rhytidodus	*1*	*0.003%*	*1*	*0.44%*
	Cicadidae		12	0.036%	2	0.89%
		Albanycada	*2*	*0.006%*	*1*	*0.44%*
	Cixiidae		1	0.003%	1	0.44%
		Cixius	*1*	*0.003%*	*1*	*0.44%*
	Coreidae		1	0.003%	1	0.44%
		Notobitus meleagris	*1*	*0.003%*	*1*	*0.44%*
	Flatidae		17	0.052%	3	1.33%
		Geisha distinctissima	*1*	*0.003%*	*1*	*0.44%*
		Metcalfa pruinosa	*15*	*0.046%*	*2*	*0.89%*
	Fulgoridae		1587	4.819%	7	3.11%
		Alphina glauca	*8*	*0.024%*	*2*	*0.89%*
		Amycle	*2*	*0.006%*	*1*	*0.44%*
		Amycle saxatilis	*1*	*0.003%*	*1*	*0.44%*
		Calyptoproctus	*1*	*0.003%*	*1*	*0.44%*
		Cyrpoptus vanduzeei	*3*	*0.009%*	*1*	*0.44%*
		Hypaepa illuminata	*3*	*0.009%*	*1*	*0.44%*
	Membracidae		6	0.018%	1	0.44%
		Calloconophora	*6*	*0.018%*	*1*	*0.44%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Hemiptera)	Pentatomidae		1	0.003%	1	0.44%
		Edessa meditabunda	*1*	*0.003%*	*1*	*0.44%*
	Reduviidae		3	0.009%	3	1.33%
		Panstrongylus herreri	*1*	*0.003%*	*1*	*0.44%*
		Rhynocoris fuscipes	*1*	*0.003%*	*1*	*0.44%*
		Zelus araneiformis	*1*	*0.003%*	*1*	*0.44%*
	Schizopteridae		1	0.003%	1	0.44%
		Pateena elimata	*1*	*0.003%*	*1*	*0.44%*
	Tessaratomidae		2	0.006%	1	0.44%
		Euthenes cupreus	*2*	*0.006%*	*1*	*0.44%*
Hymenoptera			**3076**	**9.341%**	**61**	**27.11%**
	Andrenidae		4	0.012%	2	0.89%
		Andrena dunnigi	*4*	*0.012%*	*2*	*0.89%*
	Apidae		5	0.015%	3	1.33%
		Apis cerana	*1*	*0.003%*	*1*	*0.44%*
		Diadasia ochracea	*1*	*0.003%*	*1*	*0.44%*
		Melipona capixaba	*3*	*0.009%*	*1*	*0.44%*
	Braconidae		14	0.043%	4	1.78%
		Alabagrus	*7*	*0.021%*	*1*	*0.44%*
		Chelonus	*4*	*0.012%*	*1*	*0.44%*
		Glyptapanteles	*1*	*0.003%*	*1*	*0.44%*
	Colletidae		6	0.018%	1	0.44%
		Hylaeus	*6*	*0.018%*	*1*	*0.44%*
	Crabronidae		1	0.003%	1	0.44%
		Ectemnius	*1*	*0.003%*	*1*	*0.44%*
	Formicidae		2576	7.823%	25	11.11%
		Camponotus	*16*	*0.049%*	*4*	*1.78%*
		Camponotus claviscapus occultus	*8*	*0.024%*	*1*	*0.44%*
		Crematogaster	*207*	*0.629%*	*4*	*1.78%*
		Dolichoderus taprobanae	*1*	*0.003%*	*1*	*0.44%*
		Lasius niger	*1*	*0.003%*	*1*	*0.44%*
		Pachycondyla	*95*	*0.288%*	*2*	*0.89%*
		Pachycondyla crenata	*2*	*0.006%*	*1*	*0.44%*
		Paraponera clavata	*6*	*0.018%*	*1*	*0.44%*
		Paratrechina	*1*	*0.003%*	*1*	*0.44%*
		Pheidole	*1*	*0.003%*	*1*	*0.44%*
		Pseudomyrmex	*2194*	*6.663%*	*5*	*2.22%*
		Solenopsis	*2*	*0.006%*	*1*	*0.44%*
		Tapinoma	*29*	*0.088%*	*3*	*1.33%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Hymenoptera)	Ichneumonidae		34	0.103%	6	2.67%
		Carops	*13*	*0.039%*	*1*	*0.44%*
		Sussaba erigator	*17*	*0.052%*	*4*	*1.78%*
	Megachilidae		1	0.003%	1	0.44%
		Megachile texana	*1*	*0.003%*	*1*	*0.44%*
	Mymaridae		2	0.006%	1	0.44%
		Anagrus erythroneurae	*2*	*0.006%*	*1*	*0.44%*
	Pergidae		1	0.003%	1	0.44%
		Perreyia	*1*	*0.003%*	*1*	*0.44%*
	Tenthredinidae		20	0.061%	6	2.67%
		Athalia liberta	*2*	*0.006%*	*1*	*0.44%*
		Metallus albipes	*1*	*0.003%*	*1*	*0.44%*
		Nematus	*7*	*0.021%*	*3*	*1.33%*
		Tenthredo decens	*8*	*0.024%*	*1*	*0.44%*
	Vespidae		150	0.456%	4	1.78%
		Jugurtia braunsiella	*1*	*0.003%*	*1*	*0.44%*
		Metapolybia cingulata	*9*	*0.027%*	*1*	*0.44%*
		Mischocyttarus lecointei	*2*	*0.006%*	*1*	*0.44%*
		Polistes	*5*	*0.015%*	*1*	*0.44%*
		Polistes dorsalis	*1*	*0.003%*	*1*	*0.44%*
		Polybia occidentalis	*2*	*0.006%*	*1*	*0.44%*
		Protopolybia	*3*	*0.009%*	*1*	*0.44%*
		Synoeca septentrionalis	*5*	*0.015%*	*1*	*0.44%*
Isopoda			**12**	**0.036%**	**2**	**0.89%**
	Asellidae		1	0.003%	1	0.44%
		Proasellus spelaeus	*1*	*0.003%*	*1*	*0.44%*
	Idoteidae		1	0.003%	1	0.44%
		Pentidotea stenops	*1*	*0.003%*	*1*	*0.44%*
	Ligiidae		10	0.030%	1	0.44%
		Ligidium	*10*	*0.030%*	*1*	*0.44%*
Isoptera			**2**	**0.006%**	**1**	**0.44%**
	Termitidae		2	0.006%	1	0.44%
		Odontotermes	*2*	*0.006%*	*1*	*0.44%*
Lepidoptera			**12815**	**38.916%**	**103**	**45.78%**
	Arctiidae		8	0.024%	3	1.33%
		Arctia caja	*3*	*0.009%*	*2*	*0.89%*
	Cossidae		1	0.003%	1	0.44%
		Morpheis strigifer	*1*	*0.003%*	*1*	*0.44%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Lepidoptera)	Crambidae		73	0.222%	6	2.67%
		Azochis	*1*	*0.003%*	*1*	*0.44%*
		Diaphania	*12*	*0.036%*	*2*	*0.89%*
		Lamprosema	*21*	*0.064%*	*1*	*0.44%*
		Midila daphne	*17*	*0.052%*	*1*	*0.44%*
		Phostria	*17*	*0.052%*	*1*	*0.44%*
	Elachistidae		28	0.085%	2	0.89%
		Stenoma	*1*	*0.003%*	*1*	*0.44%*
	Gelechiidae		1	0.003%	1	0.44%
		Ardozyga galactopa	*1*	*0.003%*	*1*	*0.44%*
	Geometridae		21	0.064%	6	2.67%
		Chrysocraspeda	*1*	*0.003%*	*1*	*0.44%*
		Costaconvexa centrostrigaria	*1*	*0.003%*	*1*	*0.44%*
		Dyspteris	*1*	*0.003%*	*1*	*0.44%*
		Psaliodes	*1*	*0.003%*	*1*	*0.44%*
		Xanthorhoe abrasaria	*15*	*0.046%*	*2*	*0.89%*
	Glyphiptrigidae		2	0.006%	1	0.44%
		Glyphipterix	*2*	*0.006%*	*1*	*0.44%*
	Gracillariidae		4	0.012%	3	1.33%
	Hesperiidae		88	0.267%	17	7.56%
		Achlyodes thraso	*9*	*0.027%*	*1*	*0.44%*
		Anthoptus	*1*	*0.003%*	*1*	*0.44%*
		Astraptes enotrus	*20*	*0.061%*	*1*	*0.44%*
		Astraptes tucuti	*4*	*0.012%*	*2*	*0.89%*
		Bungalotis	*4*	*0.012%*	*2*	*0.89%*
		Chrysoplectrum	*3*	*0.009%*	*1*	*0.44%*
		Dyscophellus	*24*	*0.073%*	*1*	*0.44%*
		Nyctelius	*1*	*0.003%*	*1*	*0.44%*
		Polytremis nascens	*1*	*0.003%*	*1*	*0.44%*
		Telemiades	*12*	*0.036%*	*6*	*2.67%*
		Telemiades megallus	*11*	*0.033%*	*5*	*2.22%*
	Lasiocampidae		4	0.012%	2	0.89%
		Euglyphis	*4*	*0.012%*	*2*	*0.89%*
	Limacodidae		1	0.003%	1	0.44%
		Semyra bella	*1*	*0.003%*	*1*	*0.44%*
	Lycaenidae		1	0.003%	1	0.44%
		Aricia acmon	*1*	*0.003%*	*1*	*0.44%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Lepidoptera)	Noctuidae		10	0.030%	4	1.78%
		Acronicta grisea	*1*	*0.003%*	*1*	*0.44%*
		Ochropleura implecta	*2*	*0.006%*	*1*	*0.44%*
		Spodoptera	*4*	*0.012%*	*1*	*0.44%*
		Spodoptera exempta	*1*	*0.003%*	*1*	*0.44%*
		Spodoptera frugiperda	*1*	*0.003%*	*1*	*0.44%*
	Notodontidae		19	0.058%	6	2.67%
		Hemiceras	*18*	*0.055%*	*5*	*2.22%*
		Hemiceras transducta	*15*	*0.046%*	*2*	*0.89%*
		Rosema	*1*	*0.003%*	*1*	*0.44%*
	Nymphalidae		2	0.006%	2	0.89%
		Memphis	*1*	*0.003%*	*1*	*0.44%*
		Yphthimoides leguialimai	*1*	*0.003%*	*1*	*0.44%*
	Oecophoridae		7	0.021%	5	2.22%
		Eupselia	*1*	*0.003%*	*1*	*0.44%*
		Pseudotheta syrtica	*1*	*0.003%*	*1*	*0.44%*
		Thudaca trabeata	*5*	*0.015%*	*3*	*1.33%*
	Pieridae		2	0.006%	1	0.44%
		Aphrissa statira	*2*	*0.006%*	*1*	*0.44%*
	Prodoxidae		2	0.006%	2	0.89%
		Greya politella	*1*	*0.003%*	*1*	*0.44%*
		Prodoxus quinquepunctellus	*1*	*0.003%*	*1*	*0.44%*
	Pterophoridae		1	0.003%	1	0.44%
	Pyralidae		8	0.024%	5	2.22%
		Acrobasis mniaropis	*1*	*0.003%*	*1*	*0.44%*
		Ammatucha	*1*	*0.003%*	*1*	*0.44%*
		Eccopisa effractella	*5*	*0.015%*	*2*	*0.89%*
		Nycteruetica elassota	*1*	*0.003%*	*1*	*0.44%*
	Riodinidae		1	0.003%	1	0.44%
		Calephelis	*1*	*0.003%*	*1*	*0.44%*
	Saturniidae		2	0.006%	2	0.89%
		Automeris zozimanaguana	*1*	*0.003%*	*1*	*0.44%*
		Solus parvifenestratus	*1*	*0.003%*	*1*	*0.44%*
	Sphingidae		69	0.210%	5	2.22%
		Hyles tithymali	*1*	*0.003%*	*1*	*0.44%*
		Polyptychus	*1*	*0.003%*	*1*	*0.44%*
		Pseudoangonyx excellens	*66*	*0.200%*	*3*	*1.33%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Lepidoptera)	Tortricidae		7	0.021%	6	2.67%
		Amorbia humerosana	*1*	*0.003%*	*1*	*0.44%*
		Capua hemicosmana	*1*	*0.003%*	*1*	*0.44%*
		Epiblema scudderiana	*1*	*0.003%*	*1*	*0.44%*
		Leurogyia	*3*	*0.009%*	*2*	*0.89%*
		Pammene fasciana	*1*	*0.003%*	*1*	*0.44%*
	Thyrididae		39	0.118%	1	0.44%
		Microsca	*39*	*0.118%*	*1*	*0.44%*
Mantodea			**187**	**0.568%**	**9**	**4.00%**
	Liturgusidae		69	0.210%	5	2.22%
		Liturgusa maya	*69*	*0.210%*	*5*	*2.22%*
	Mantidae		110	0.334%	5	2.22%
		Choeradodis rhombicollis	*2*	*0.006%*	*1*	*0.44%*
		Oromantis	*53*	*0.161%*	*1*	*0.44%*
	Sibyliidae		1	0.003%	1	0.44%
		Sibylla	*1*	*0.003%*	*1*	*0.44%*
Megaloptera			**1**	**0.003%**	**1**	**0.44%**
Odonata			**1**	**0.003%**	**1**	**0.44%**
	Platystictidae		1	0.003%	1	0.44%
		Platysticta greeni	*1*	*0.003%*	*1*	*0.44%*
Orthoptera			**660**	**2.004%**	**37**	**16.44%**
	Acrididae		422	1.282%	24	10.67%
		Achurum carinatum	*3*	*0.009%*	*1*	*0.44%*
		Austracris guttulosa	*1*	*0.003%*	*1*	*0.44%*
		Baeacris	*1*	*0.003%*	*1*	*0.44%*
		Catantops	*28*	*0.085%*	*4*	*1.78%*
		Dichroplus	*2*	*0.006%*	*2*	*0.89%*
		Dicrhoplus silveiraguidoi	*1*	*0.003%*	*1*	*0.44%*
		Dicrhoplus vittiger	*1*	*0.003%*	*1*	*0.44%*
		Emeiacris maculata	*1*	*0.003%*	*1*	*0.44%*
		Goniaea vocans	*1*	*0.003%*	*1*	*0.44%*
		Hesperotettix viridis	*14*	*0.043%*	*3*	*1.33%*
		Melanoplus	*2*	*0.006%*	*1*	*0.44%*
		Melanoplus femurnigrum	*1*	*0.003%*	*1*	*0.44%*
		Morphacris fasciata	*1*	*0.003%*	*1*	*0.44%*
		Pardalophora apiculata	*8*	*0.024%*	*1*	*0.44%*
		Schistocerca	*9*	*0.027%*	*4*	*1.78%*
		Schistocerca cancellata	*2*	*0.006%*	*1*	*0.44%*
		Schistocerca flavofasciata	*1*	*0.003%*	*1*	*0.44%*
		Scotussa bracyptera	*2*	*0.006%*	*2*	*0.89%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Orthoptera)	(Acrididae)	*Sphingonotus canariensis*	*1*	*0.003%*	*1*	*0.44%*
		Stenocatantops splendens	*1*	*0.003%*	*1*	*0.44%*
	Gryllidae		61	0.185%	7	3.11%
		Agnotecous	1	0.003%	1	0.44%
		Amphiacusta	53	0.161%	4	1.78%
		Gryllotalpa pluvialis	1	0.003%	1	0.44%
		Gryllus	2	0.006%	2	0.89%
		Gryllus bimaculatus	1	0.003%	1	0.44%
		Loxoblemmus doenitzi	1	0.003%	1	0.44%
	Morabidae		1	0.003%	1	0.44%
		Warramaba	*1*	*0.003%*	*1*	*0.44%*
	Stenopelmatidae		1	0.003%	1	0.44%
		Stenopelmatus	*1*	*0.003%*	*1*	*0.44%*
	Tettigoniidae		2	0.006%	1	0.44%
		Eupholidoptera	*2*	*0.006%*	*1*	*0.44%*
Pantopoda			**1**	**0.003%**	**1**	**0.44%**
Parasitiformes			**5**	**0.015%**	**5**	**2.22%**
	Ixodidae		1	0.003%	1	0.44%
		Amplyomma limbatum	*1*	*0.003%*	*1*	*0.44%*
	Parasitidae		1	0.003%	1	0.44%
Pendunculata			**104**	**0.316%**	**3**	**1.33%**
	Lepadidae		104	0.316%	3	1.33%
		Lepas anatifera	*104*	*0.316%*	*3*	*1.33%*
Phasmatodea			**44**	**0.134%**	**8**	**3.56%**
	Diapheromeridae		3	0.009%	1	0.44%
		Diapheromera femorata	*3*	*0.009%*	*1*	*0.44%*
	Phasmatidae		14	0.043%	4	1.78%
		Phobaeticus serratipes	*14*	*0.043%*	*4*	*1.78%*
Plecoptera			**12**	**0.036%**	**6**	**2.67%**
	Perlodidae		8	0.024%	5	2.22%
		Isogenoides frontalis	*8*	*0.024%*	*5*	*2.22%*
Poduromorpha			**74**	**0.225%**	**6**	**2.67%**
	Hypogastruridae		52	0.158%	3	1.33%
		Xenylla humicola	*52*	*0.158%*	*3*	*1.33%*
	Neanuridae		1	0.003%	1	0.44%
		Lobella	*1*	*0.003%*	*1*	*0.44%*
	Onychiuridae		1	0.003%	1	0.44%
		Megaphorura arctica	*1*	*0.003%*	*1*	*0.44%*
	Tullbergiidae		1	0.003%	1	0.44%
		Mesaphorura	*1*	*0.003%*	*1*	*0.44%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
Sarcoptiformes			**169**	**0.513%**	**6**	**2.67%**
	Acaridae		32	0.097%	2	0.89%
		Caloglyphus berlesei	*11*	*0.033%*	*2*	*0.89%*
	Analgidae		5	0.015%	1	0.44%
		Analges	*5*	*0.015%*	*1*	*0.44%*
	Chaetodactylidae		1	0.003%	1	0.44%
	Proctophyllodidae		1	0.003%	1	0.44%
		Proctophyllodes	*1*	*0.003%*	*1*	*0.44%*
	Psoroptidae		2	0.006%	1	0.44%
		Caparinia	1	0.003%	1	0.44%
	Tectocepheidae		1	0.003%	1	0.44%
		Tectocepheus	*1*	*0.003%*	*1*	*0.44%*
	Syringobiidae		1	0.003%	1	0.44%
		Syringobia longipenis	*1*	*0.003%*	*1*	*0.44%*
Scolopendromorpha			**3**	**0.009%**	**2**	**0.89%**
	Scolopendridae		3	0.009%	2	0.89%
		Rhysida	*1*	*0.003%*	*1*	*0.44%*
Scorpiones			**5**	**0.015%**	**3**	**1.33%**
	Buthidae		3	0.009%	2	0.89%
		Buthus occitanus	*2*	*0.006%*	*1*	*0.44%*
		Odontobuthus doriae	*1*	*0.003%*	*1*	*0.44%*
Sessilia			**20**	**0.061%**	**3**	**1.33%**
	Balanidae		3	0.009%	2	0.89%
		Balanus glandula	*3*	*0.009%*	*2*	*0.89%*
	Chthamalidae		17	0.052%	2	0.89%
		Chthamalus southwardorum	*17*	*0.052%*	*2*	*0.89%*
Stomatopoda			**2**	**0.006%**	**1**	**0.44%**
	Gonadactylidae		2	0.006%	1	0.44%
		Gonodactylellus erdmanni	*2*	*0.006%*	*1*	*0.44%*
Syncarida			**527**	**1.600%**	**2**	**0.89%**
	Anaspididae		527	1.600%	2	0.89%
		Anaspides tasmaniae	*527*	*1.600%*	*2*	*0.89%*
Tricoptera			**6**	**0.018%**	**7**	**3.11%**
	Brachycentridae		1	0.003%	1	0.44%
		Micrasema bactro	*1*	*0.003%*	*1*	*0.44%*
	Calamoceratidae		1	0.003%	1	0.44%
		Phylloicus aeneus	*1*	*0.003%*	*1*	*0.44%*
	Ecnomidae		1	0.003%	1	0.44%
		Agmina	*1*	*0.003%*	*1*	*0.44%*

Order	Family	Genus and Species	Number OTUs	Percent relative abundance	Number of samples	Frequency of occurrence
(Tricoptera)	Glossosomatidae		1	0.003%	1	0.44%
		Agapetus	*1*	*0.003%*	*1*	*0.44%*
	Hydropsychidae		1	0.003%	1	0.44%
		Macrostemum carolina	*1*	*0.003%*	*1*	*0.44%*
	Philorheithridae		1	0.003%	1	0.44%
		Philorheithrus aliciae	*1*	*0.003%*	*1*	*0.44%*
Trombidiformes			**114**	**0.346%**	**4**	**1.78%**
	Eupodidae		1	0.003%	1	0.44%
	Rhagidiidae		1	0.003%	1	0.44%
	Tarsonemidae		6	0.018%	1	0.44%

APPENDIX D

RELATIVE ABUNDANCE OF BACTERIA IN THE WHITE-FACED CAPUCHIN GUT

Phylum	Order	Family	Genus	Relative Abundance
Acidobacteria	Acidobacteria Gp1 incertae sedis	Acidobacteria Gp1 incertae sedis	Acidobacteria Gp1 incertae sedis	0.07839%
Acidobacteria	Acidobacteria Gp25 incertae sedis	Acidobacteria Gp25 incertae sedis	Acidobacteria Gp25 incertae sedis	0.00537%
Acidobacteria	Acidobacteria Gp3 incertae sedis	Acidobacteria Gp3 incertae sedis	Acidobacteria Gp3 incertae sedis	0.00011%
Acidobacteria	Acidobacteria Gp4 incertae sedis	Acidobacteria Gp4 incertae sedis	Acidobacteria Gp4 incertae sedis	0.00133%
Acidobacteria	Acidobacteria Gp5 incertae sedis	Acidobacteria Gp5 incertae sedis	Acidobacteria Gp5 incertae sedis	0.01557%
Acidobacteria	Holophagales	Holophagaceae	Geothrix	0.00138%
Actinobacteria	Acidimicrobiales	Acidimicrobineae incertae sedis	Aciditerrimonas	0.00031%
Actinobacteria	Actinomycetales	Beutenbergiaceae	Salana	0.00386%
Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium	0.04708%
Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	0.00021%
Actinobacteria	Actinomycetales	Cryptosporangiaceae	Cryptosporangium	0.00085%
Actinobacteria	Actinomycetales	Geodermatophilaceae	Blastococcus	0.00007%
Actinobacteria	Actinomycetales	Intrasporangiaceae	Marihabitans	0.00023%
Actinobacteria	Actinomycetales	Kineosporiaceae	Kineococcus	0.01116%
Actinobacteria	Actinomycetales	Kineosporiaceae	Kineosporia	0.00046%
Actinobacteria	Actinomycetales	Microbacteriaceae	Agrococcus	0.00478%
Actinobacteria	Actinomycetales	Microbacteriaceae	Curtobacterium	0.01883%
Actinobacteria	Actinomycetales	Microbacteriaceae	Microbacterium	0.00185%
Actinobacteria	Actinomycetales	Micrococcaceae	Nesterenkonia	0.00191%
Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	0.01069%
Actinobacteria	Actinomycetales	Micromonosporaceae	Pilimelia	0.00278%
Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	0.35728%
Actinobacteria	Actinomycetales	Nocardiaceae	Gordonia	0.00063%
Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	0.00007%
Actinobacteria	Actinomycetales	Nocardiaceae	Williamsia	0.00055%
Actinobacteria	Actinomycetales	Nocardioidaceae	Aeromicrobium	0.03847%
Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	0.00082%
Actinobacteria	Actinomycetales	Propionibacteriaceae	Propionibacterium	0.04499%
Actinobacteria	Actinomycetales	Pseudonocardiaceae	Actinomycetospora	0.00022%
Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia	0.00218%
Actinobacteria	Actinomycetales	Tsukamurellaceae	Tsukamurella	0.00076%
Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00664%

Phylum	Order	Family	Genus	Relative Abundance
Actinobacteria	Coriobacteriales	Coriobacteriaceae	Eggerthella	0.01114%
Actinobacteria	Coriobacteriales	Coriobacteriaceae	Slackia	0.00004%
Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	0.00023%
Actinobacteria	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	0.00351%
Actinobacteria	Thermoleophilales	Thermoleophilaceae	Thermoleophilum	0.04171%
Aquificae	Aquificales	Hydrogenothermaceae	Hydrogenothermus	0.00278%
Armatimonadetes	Armatimonadetes Gp5 incertae sedis	Armatimonadetes Gp5 incertae sedis	Armatimonadetes Gp5 incertae sedis	0.00356%
Bacteroidetes	Bacteroidales	Bacteroidaceae	Bacteroides	0.57858%
Bacteroidetes	Bacteroidales	Porphyromonadaceae	Barnesiella	0.00549%
Bacteroidetes	Bacteroidales	Porphyromonadaceae	Dysgonomonas	0.00149%
Bacteroidetes	Bacteroidales	Porphyromonadaceae	Porphyromonas	0.06993%
Bacteroidetes	Bacteroidales	Prevotellaceae	Hallella	0.30181%
Bacteroidetes	Bacteroidales	Prevotellaceae	Paraprevotella	1.42884%
Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	0.08595%
Bacteroidetes	Bacteroidales	Prevotellaceae	Xylanibacter	10.43606%
Bacteroidetes	Bacteroidetes incertae sedis	Bacteroidetes incertae sedis	Ohtaekwangia	0.10778%
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Actibacter	0.01909%
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	0.02211%
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	0.02165%
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Cloacibacterium	0.01129%
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0.06328%
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Pseudozobellia	0.00021%
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Wautersiella	0.02577%
Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Chitinophaga	0.00013%
Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Ferruginibacter	0.00328%
Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Lacibacter	0.00010%
Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Niabella	0.00119%
Bacteroidetes	Sphingobacteriales	Cytophagaceae	Dyadobacter	0.00022%
Bacteroidetes	Sphingobacteriales	Cytophagaceae	Microscilla	0.01119%
Bacteroidetes	Sphingobacteriales	Cytophagaceae	Siphonobacter	0.01133%
Bacteroidetes	Sphingobacteriales	Cytophagaceae	Spirosoma	0.00192%
Bacteroidetes	Sphingobacteriales	Flammeovirgaceae	Cesiribacter	0.00013%
Bacteroidetes	Sphingobacteriales	Saprospiraceae	Aureispira	0.00023%
Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	0.00145%
Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Olivibacter	0.00002%
Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	0.00428%
Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Pseudosphingobacterium	0.00368%
Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	0.05897%

Phylum	Order	Family	Genus	Relative Abundance
Chloroflexi	Caldilineales	Caldilineaceae	Caldilinea	0.07151%
Cyanobacteria_Chloroplast	Chloroplast incertae sedis	Chloroplast	Bangiophyceae	0.01561%
Cyanobacteria_Chloroplast	Chloroplast incertae sedis	Chloroplast	Streptophyta	4.40633%
Fibrobacteres	Fibrobacterales	Fibrobacteraceae	Fibrobacter	0.01393%
Firmicutes	Bacillales	Bacillaceae 1	Bacillus	0.18002%
Firmicutes	Bacillales	Bacillales Incertae Sedis XI	Gemella	0.00039%
Firmicutes	Bacillales	Paenibacillaceae 1	Paenibacillus	0.00231%
Firmicutes	Bacillales	Paenibacillaceae 1	Saccharibacillus	0.00037%
Firmicutes	Bacillales	Staphylococcaceae	Staphylococcus	0.00019%
Firmicutes	Clostridiales	Clostridiaceae 1	Anaerosporobacter	0.00194%
Firmicutes	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto	0.09401%
Firmicutes	Clostridiales	Clostridiaceae 1	Sarcina	0.37655%
Firmicutes	Clostridiales	Lachnospiraceae	Anaerostipes	0.01498%
Firmicutes	Clostridiales	Lachnospiraceae	Catonella	0.00005%
Firmicutes	Clostridiales	Lachnospiraceae	Cellulosilyticum	0.00103%
Firmicutes	Clostridiales	Lachnospiraceae	Clostridium XlVa	16.42745%
Firmicutes	Clostridiales	Lachnospiraceae	Clostridium XlVb	0.55511%
Firmicutes	Clostridiales	Lachnospiraceae	Coprococcus	0.03137%
Firmicutes	Clostridiales	Lachnospiraceae	Dorea	0.00447%
Firmicutes	Clostridiales	Lachnospiraceae	Lachnospiracea incertae sedis	0.37150%
Firmicutes	Clostridiales	Lachnospiraceae	Marvinbryantia	0.01248%
Firmicutes	Clostridiales	Lachnospiraceae	Moryella	0.00004%
Firmicutes	Clostridiales	Lachnospiraceae	Robinsoniella	0.01285%
Firmicutes	Clostridiales	Lachnospiraceae	Sporobacterium	0.00055%
Firmicutes	Clostridiales	Peptostreptococcaceae	Clostridium XI	0.10384%
Firmicutes	Clostridiales	Ruminococcaceae	Anaerotruncus	0.00765%
Firmicutes	Clostridiales	Ruminococcaceae	Butyricicoccus	0.00914%
Firmicutes	Clostridiales	Ruminococcaceae	Clostridium IV	0.01867%
Firmicutes	Clostridiales	Ruminococcaceae	Flavonifractor	0.00008%
Firmicutes	Clostridiales	Ruminococcaceae	Subdoligranulum	0.00033%
Firmicutes	Erysipelotrichales	Erysipelotrichaceae	Clostridium XVIII	0.06287%
Firmicutes	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae incertae sedis	0.00113%
Firmicutes	Erysipelotrichales	Erysipelotrichaceae	Turicibacter	0.00668%
Firmicutes	Lactobacillales	Aerococcaceae	Abiotrophia	0.03146%
Firmicutes	Lactobacillales	Aerococcaceae	Aerococcus	0.00092%
Firmicutes	Lactobacillales	Aerococcaceae	Eremococcus	0.00004%
Firmicutes	Lactobacillales	Carnobacteriaceae	Atopobacter	0.01964%

Phylum	Order	Family	Genus	Relative Abundance
Firmicutes	Lactobacillales	Enterococcaceae	Enterococcus	0.24095%
Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus	0.28448%
Firmicutes	Lactobacillales	Lactobacillaceae	Paralactobacillus	0.66669%
Firmicutes	Lactobacillales	Leuconostocaceae	Leuconostoc	0.00818%
Firmicutes	Lactobacillales	Leuconostocaceae	Weissella	0.65107%
Firmicutes	Lactobacillales	Streptococcaceae	Lactococcus	1.24475%
Firmicutes	Lactobacillales	Streptococcaceae	Streptococcus	13.24339%
Firmicutes	Selenomonadales	Veillonellaceae	Dialister	0.18659%
Firmicutes	Selenomonadales	Veillonellaceae	Megamonas	5.60799%
Firmicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.92977%
Firmicutes	Selenomonadales	Veillonellaceae	Veillonella	0.11666%
Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	0.05775%
Fusobacteria	Fusobacteriales	Leptotrichiaceae	Leptotrichia	0.18131%
Fusobacteria	Fusobacteriales	Leptotrichiaceae	Sebaldella	0.00023%
Fusobacteria	Fusobacteriales	Leptotrichiaceae	Streptobacillus	0.01072%
Proteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	1.22128%
Proteobacteria	Alteromonadales	Shewanellaceae	Shewanella	0.00342%
Proteobacteria	Bdellovibrionales	Bdellovibrionaceae	Vampirovibrio	0.00018%
Proteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter	0.00112%
Proteobacteria	Burkholderiales	Alcaligenaceae	Advenella	0.09010%
Proteobacteria	Burkholderiales	Alcaligenaceae	Bordetella	0.00007%
Proteobacteria	Burkholderiales	Alcaligenaceae	Castellaniella	0.00151%
Proteobacteria	Burkholderiales	Alcaligenaceae	Derxia	0.03468%
Proteobacteria	Burkholderiales	Alcaligenaceae	Parapusillimonas	0.00367%
Proteobacteria	Burkholderiales	Alcaligenaceae	Pusillimonas	0.01995%
Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	0.35937%
Proteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	0.00739%
Proteobacteria	Burkholderiales	Burkholderiaceae	Pandoraea	0.04136%
Proteobacteria	Burkholderiales	Burkholderiales incertae sedis	Aquabacterium	0.00056%
Proteobacteria	Burkholderiales	Burkholderiales incertae sedis	Ideonella	0.00048%
Proteobacteria	Burkholderiales	Burkholderiales incertae sedis	Methylibium	0.00358%
Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	0.00524%
Proteobacteria	Burkholderiales	Comamonadaceae	Brachymonas	0.00009%
Proteobacteria	Burkholderiales	Comamonadaceae	Diaphorobacter	0.01668%
Proteobacteria	Burkholderiales	Comamonadaceae	Pelomonas	0.00875%
Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	0.00003%
Proteobacteria	Burkholderiales	Comamonadaceae	Simplicispira	0.00272%

Phylum	Order	Family	Genus	Relative Abundance
Proteobacteria	Burkholderiales	Oxalobacteraceae	Collimonas	0.00641%
Proteobacteria	Burkholderiales	Oxalobacteraceae	Undibacterium	0.00307%
Proteobacteria	Burkholderiales	Sutterellaceae	Parasutterella	0.00070%
Proteobacteria	Burkholderiales	Sutterellaceae	Sutterella	6.13140%
Proteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.00284%
Proteobacteria	Campylobacterales	Helicobacteraceae	Helicobacter	0.00526%
Proteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	0.00985%
Proteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	0.00433%
Proteobacteria	Chromatiales	Chromatiaceae	Thiobaca	0.00143%
Proteobacteria	Desulfarculales	Desulfarculaceae	Desulfarculus	0.00043%
Proteobacteria	Desulfurellales	Desulfurellaceae	Hippea	0.00836%
Proteobacteria	Desulfuromonadales	Desulfuromonadaceae	Malonomonas	0.01024%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter	1.03522%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	0.69285%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia	0.18204%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia shigella	4.06830%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	0.49364%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Kluyvera	0.22551%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Morganella	0.04360%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	2.21673%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Pectobacterium	0.07737%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Serratia	0.59520%
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Tatumella	1.01749%
Proteobacteria	Gammaproteobacteria incertae sedis	Gammaproteobacteria incertae sedis	Orbus	0.00419%
Proteobacteria	Legionellales	Coxiellaceae	Aquicella	0.00893%
Proteobacteria	Legionellales	Coxiellaceae	Coxiella	0.70084%
Proteobacteria	Legionellales	Legionellaceae	Legionella	0.00341%
Proteobacteria	Myxococcales	Kofleriaceae	Kofleria	0.00745%
Proteobacteria	Myxococcales	Nannocystaceae	Nannocystis	0.00013%
Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	0.00015%
Proteobacteria	Neisseriales	Neisseriaceae	Kingella	0.04799%
Proteobacteria	Neisseriales	Neisseriaceae	Neisseria	0.54847%
Proteobacteria	Neisseriales	Neisseriaceae	Paludibacterium	0.00172%
Proteobacteria	Neisseriales	Neisseriaceae	Simonsiella	0.00038%
Proteobacteria	Nitrosomonadales	Nitrosomonadaceae	Nitrosospira	0.01210%
Proteobacteria	Oceanospirillales	Halomonadaceae	Carnimonas	0.00932%
Proteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00018%
Proteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus	7.54239%

Phylum	Order	Family	Genus	Relative Abundance
Proteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella	0.04971%
Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0.15235%
Proteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	0.00003%
Proteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	0.00287%
Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	2.39378%
Proteobacteria	Rhizobiales	Aurantimonadaceae	Aurantimonas	0.05056%
Proteobacteria	Rhizobiales	Beijerinckiaceae	Beijerinckia	0.00657%
Proteobacteria	Rhizobiales	Beijerinckiaceae	Methylocapsa	0.00421%
Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Afipia	0.15629%
Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	0.00472%
Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Salinarimonas	0.00972%
Proteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	0.07555%
Proteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	0.00176%
Proteobacteria	Rhizobiales	Hyphomicrobiaceae	Prosthecomicrobium	0.04741%
Proteobacteria	Rhizobiales	Methylobacteriaceae	Meganema	0.00364%
Proteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	0.10812%
Proteobacteria	Rhizobiales	Phyllobacteriaceae	Nitratireductor	0.05753%
Proteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	1.09748%
Proteobacteria	Rhizobiales	Rhizobiaceae	Sinorhizobium	0.00005%
Proteobacteria	Rhizobiales	Rhodobiaceae	Tepidamorphus	0.00346%
Proteobacteria	Rhodobacterales	Rhodobacteraceae	Ketogulonicigenium	0.00118%
Proteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	0.00033%
Proteobacteria	Rhodobacterales	Rhodobacteraceae	Rubellimicrobium	0.00072%
Proteobacteria	Rhodocyclales	Rhodocyclaceae	Azospira	0.10557%
Proteobacteria	Rhodocyclales	Rhodocyclaceae	Sulfuritalea	0.00017%
Proteobacteria	Rhodocyclales	Rhodocyclaceae	Uliginosibacterium	0.00086%
Proteobacteria	Rhodospirillales	Acetobacteraceae	Gluconobacter	0.02915%
Proteobacteria	Rhodospirillales	Acetobacteraceae	Granulibacter	0.03094%
Proteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas	0.08720%
Proteobacteria	Rhodospirillales	Acetobacteraceae	Tanticharoenia	0.01297%
Proteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	0.00166%
Proteobacteria	Rhodospirillales	Rhodospirillaceae	Dongia	0.00027%
Proteobacteria	Rickettsiales	Anaplasmataceae	Neorickettsia	0.00020%
Proteobacteria	Rickettsiales	Mitochondria	Mitochondria incertae sedis	0.54506%
Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sandaracinobacter	0.00270%
Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	0.00085%
Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0.23478%
Proteobacteria	Syntrophobacterales	Syntrophaceae	Smithella	0.01977%

Phylum	Order	Family	Genus	Relative Abundance
Proteobacteria	Thiotrichales	Piscirickettsiaceae	Sulfurivirga	0.00003%
Proteobacteria	Thiotrichales	Thiotrichales incertae sedis	Caedibacter	0.00006%
Proteobacteria	Xanthomonadales	Xanthomonadaceae	Aquimonas	0.00005%
Proteobacteria	Xanthomonadales	Xanthomonadaceae	Frateuria	0.02788%
Proteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	0.05128%
Proteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	1.23127%
Proteobacteria	Xanthomonadales	Xanthomonadaceae	Thermomonas	0.00247%
Proteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	2.94344%
Tenericutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.02834%
Tenericutes	Anaeroplasmatales	Anaeroplasmataceae	Asteroleplasma	0.00032%
Tenericutes	Entomoplasmatales	Spiroplasmataceae	Spiroplasma	0.00099%
Verrucomicrobia	Opitutales	Opitutaceae	Opitutus	0.01891%